



*Acta*

# OTO-LARYNGOLOGICA

VOL. 78 · JULY-AUGUST 1974 · No. 1-2

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Despite the potentially serious consequences of A V no experimental study of the phenomenon in man has been published so far. The actual mechanism has been a matter of dispute for years, and opinions differ as to how pressure changes might cause vertigo. Most authors agree that a pressure difference between the middle ear cavity and the atmosphere in some way causes the vertigo. It is also generally assumed that the vertigo is caused by a relative overpressure in the middle ear since most subjects who have some experience of A V have observed that it occurs when the middle ear pressure has been increased indirectly by lowering the ambient pressure at ascents or directly by blowing against a clamped nose (Valsalva's manoeuvre).

The mechanism by which pressure affects the inner ear is, however, still unknown. Vestibular stimulation due to pressure changes in the middle ear was early observed in subjects with a labyrinthine fistula. In 1908 Bárány introduced the "fistula test", by which such fistulas could be diagnosed by compression and aspiration in the external ear canal in subjects having an eardrum perforation due to chronic otitis media. In 1919 Karlfors & Nylen (Nylen, 1923) introduced the term "pseudo-fistula symptom" for the vertigo which could be elicited in some subjects although a labyrinthine fistula could be found. The phenomenon was, however, noticed long before by many authors who had observed "fistula symptoms without fistula". These symptoms were often seen in subjects with lues and explained as due to an abnormal mobility of the stapes footplate caused by luetic perostitis around the stapes. That displacement of the stapes footplate could cause vestibular stimulation was also noted by House (1968) when pushing down the stapes with a needle during ear surgery. As for the mechanism through which pressure changes may induce vertigo in normal subjects it has been suggested that pressure somehow induces an unusual large displacement of the stapes footplate, and thus causes inner ear fluid to flow and stimulate the vestibular receptors (Fields, 1958; Heller, 1960; Hilger, 1964). Some

authors consider the mechanism by which vertigo is elicited akin to the fistula mechanism. Thus Melvill Jones (1957) and Benson (1965) suggest that the bony wall separating the middle ear cavity from the horizontal canal may thin in certain subjects as to become deformed when the middle ear pressure differs from ambient pressure.

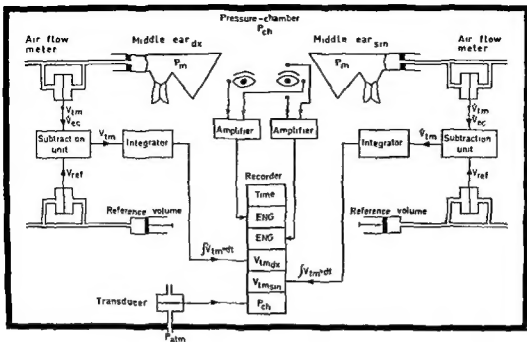
Furthermore, the observations of vertigo under field conditions appear frequently to be long (several minutes) to be caused by movements of the stapes (Lundgren, 1965; Varmann & Bradley, 1970; Lundgren et al., 1971). However, from these and other studies it appears that A V most frequently occurs during ascents both in flight and diving, i.e. by a fall in ambient pressure with a resultant relative overpressure in the middle ears. This overpressure seems to be crucial for eliciting A V. There is no information in the literature about the amount of overpressure necessary for eliciting A V but it is well known that most pilots and divers at ascents usually do not clear their ears actively but wait for their ears to be cleared passively when the relative overpressure has increased enough to force the Eustachian tubes open. According to this it seems to be of essential interest to find out whether the forcing pressure is strong enough to cause vertigo in certain subjects or if other mechanisms are involved in the phenomenon.

The aims of the present work were (i) to determine the level of relative overpressure in the middle ears that might result from ambient pressure decrease, i.e. the level of overpressure necessary to force the Eustachian tubes open (forcing pressure), (ii) to see if such overpressure can cause A V in normal subjects, and (iii) to investigate, by means of ENG, if experimentally elicited A V might be of vestibular origin.

## EQUIPMENT AND METHOD

The following symbols are used

- Patm, atmosphere on ground
- Pm, pressure in the middle ear
- P<sub>tm</sub>, pressure gradient across the eardrum



Outline of the equipment used for recordings of displacement of the eardrums ( $V_{tm}$ ) at changing ambient pressure ( $P_{ch}$ ). For details, see text

pressure in the chamber, i.e. ambient pressure

pressure in the external ear canal

volume of the air-filled middle ear space

volume displacement of the eardrum in relation to its neutral position

$c$ , volume of the mucous membrane lining the middle ear space

airflow through the resistor of the ear canal flowmeter caused by the volume displacement of the eardrum

airflow through the resistor of the ear canal flowmeter, caused by expansion or compression of the gas volume in the external ear canal and in the flowmeter system by changing the ambient pressure

airflow through the resistor of the reference flowmeter, caused by expansion or compression of the gas volume in the reference system by changing the ambient pressure

$\Delta$  before the symbol indicates a change of the variable. Pressure is expressed in cm H<sub>2</sub>O, volume in microlitre,  $\mu$ l, or millilitre, ml, and airflow in microlitre/sec,  $\mu$ l/sec

$P_m$ ,  $P_{ch}$  and  $P_{ec}$  are relative to atmosphere pressure on the ground

$P_{m_d}$  middle ear pressure minus the pressure of saturated water vapour at 37°C, this being regarded as non-compressible in connection with Boyle's law

$K_{muc}$ , volume-pressure relation of the middle ear mucosa (compliance), at the experimental pressure conditions of the present study

Fig 1 gives an outline of the equipment used for the recordings. A pressure chamber was used in which it is possible to simulate "ascents" and "descents" of 90 cm H<sub>2</sub>O in 25 sec, with a constant rate of pressure change during 90% of the time. The subjects were placed in the

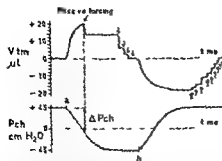


Fig. 2 Recording of eardrum volume displacement ( $V_{tm}$  in  $\mu$ l) and chamber pressure ( $P_{ch}$  in  $\text{cm H}_2\text{O}$ ). Eardrum movement outwards (+) and inwards (-). Each examination starts with a simulated descent with a pressure increase of  $+45 \text{ cm H}_2\text{O}$ . It is then checked that the eardrum is in its neutral position ( $V_{tm}=0$ ) after active equilibration. The simulated ascent then starts (a) with a pressure decrease of  $90 \text{ cm H}_2\text{O}$ . The Eustachian tube is opened passively (passive forcing), and the eardrum moves inwards to the neutral position when the middle ear pressure is equilibrated to the chamber pressure by deglutitions (arrows). When the chamber pressure is increased (b) the eardrum moves outwards, and when the middle ear pressure is equilibrated by deglutitions (arrows) the eardrum again moves to its neutral position.

chamber and exposed to pressure changes. A polyethylene catheter with its end through a rubber disc was inserted into the inner bony part of the external ear canal connecting the space between the eardrum and the rubber disc with the measuring device. A method was used, described by Ingelstedt et al (1967) and Elner et al (1971), by which it is possible to record the displacement of the eardrum ( $V_{tm}$ ) in relation to its neutral position when the ambient pressure ( $P_{ch}$ ) is changed. The method implies free air communication between the outside of the eardrum and the ambient pressure and is based on recording, by means of a flowmeter, the airflow caused by the eardrum displacement ( $V_{tm}$ ). The flow  $V_{ec}$  must be eliminated for an isolated recording of  $V_{tm}$  during ambient pressure changes. This elimination is possible by using an identical flowmeter system with an adjustable reference volume. After this elimination the flow signal ( $V_{tm}$ ) is integrated and  $V_{tm}$  is recorded. From these recordings of  $V_{tm}$  and  $P_{ch}$  it is possible to determine the ambient pressure change ( $\Delta P_{ch}$ ) necessary to cause a passive opening of the Eustachian tubes (passive

forcing). Fig. 2 gives an example of a recording. For details of the method and the equipment Ingelstedt et al (1967) and Elner et al (1971).

Simultaneously with the recordings of  $P_{ch}$  and  $V_{tm}$ , recording of eye-movements by means of electronystagmography (ENG) was performed.

and performance.

Subject's eyes open in order to get a recording of any vestibularly induced eye-movement (Tjeström, 1973). In the ensuing series of experiments only horizontal leads were used, as vertical nystagmus did not appear in the first series. Specially devised electrodes for ENG-recording were used (Siemens-Elma AB, Sweden).

Calibration of the equipment for recording  $V_{tm}$  and  $P_{ch}$  was performed before and after every test according to the original method. Eye-movements were calibrated before and after every test for  $30^\circ$  in each direction.

Estimation of the forcing pressure as equivalent to  $\Delta P_{ch}$  (the pressure change in the chamber during the ascent recorded at the moment of passive opening of the tube) is only approximate. For a correct determination of the pressure gradient across the tube, i.e. the pressure gradient across the eardrum ( $P_{tm}$ ) necessary to force the tube open passively, knowledge is required of the middle ear pressure ( $P_m$ ) as well as of the ambient pressure ( $P_{ch}$ ) at the moment of passive opening. Before each test it was checked that the eardrum was in its neutral position which means that  $P_{ch}=P_m$ , i.e.  $P_{tm}=0$ . At the moment of passive forcing the pressure gradient across the eardrum ( $P_{tm}$ ) is equal to the difference between the pressure change in the chamber ( $\Delta P_{ch}$ ) and the pressure change in the middle ear ( $\Delta P_m$ ), i.e.

$$P_{tm} = \Delta P_{ch} - \Delta P_m \quad (\text{eq. 1})$$

Fig. 3 gives an outline of the different variables involved. Determination of the middle ear pressure change ( $\Delta P_m$ ) during changing ambient pressure ( $\Delta P_{ch}$ ) requires knowledge of the following factors:

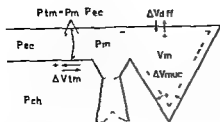


Fig. 3 Middle ear model with its variables. For explanation of symbols see text.

m,  $\Delta V_{tm}$  and  $\Delta V_{muc}$

Since changes in the middle ear pressure cause change in the volume of the vessels lining the panic cavity, and thus affect the middle ear pressure, this volume factor ( $V_{muc}$ ) has to be determined.

When these factors are known it is possible to calculate the middle ear pressure change ( $\Delta P_m$ ) at changing ambient pressure by using the following equation derived from Boyle's law (Ingelstedt et al., 1967)

$$\Delta P_m = \frac{\Delta P_{ch} \times K_{muc} + \Delta V_{tm}}{K_{muc} + \frac{V_m}{P_{m_e}}} \quad (\text{eq. 2})$$

$K_{muc}$  = the volume pressure relation (compliance) of the middle ear mucosa (see text)

The volume of the middle ear may be determined directly via a perforation of the eardrum (Flisberg et al., 1963, Andreasson 1973), or indirectly across an intact eardrum (Elnér et al., 1971a). It has been shown that there is a linear relation between a roentgenological planimetric determination of the area ( $A_m$ ) and a direct volume determination ( $V_m$ ) of the air-filled middle ear system (Flisberg & Zsigmond, 1965, Andreasson, 1973). According to Andreasson this relation was  $V_m = 0.49 \times A_m - 0.38$  in a study of 9 subjects with a traumatic perforation of the eardrum.

In the present study, the middle ear volume was determined indirectly by measuring the area of the ear cell system on X-ray films (projection II according to Runström 1933), applying a planimeter method similar to that introduced by Diamant (1940). This is the same method as that

used by Flisberg & Zsigmond (1965) and Andreasson (1973).

The volume displacement of the eardrum was determined by the method devised by Ingelstedt et al. (1967) and Elnér et al. (1971a).

The volume variation of the mucosa ( $\Delta V_{muc}$ ) has been studied by Andreasson et al. (1974), and the volume pressure relation (compliance,  $K_{muc}$ ) was calculated at 0.5  $\mu\text{l}/\text{cm H}_2\text{O}$  in the experimental conditions used in the present study.

The diffusion of gas from the middle ear ( $\Delta V_{diff}$ ) also affects the middle ear pressure. Since the diffusion is slow (0.5–1.0  $\mu\text{l}/\text{min}$ , Elnér, 1971, Ingelstedt & Jonson, 1967) this factor is negligible in studies on rapid events as in the present study with time periods of only about 20 sec.

## MATERIAL

The material consisted of 79 subjects aged between 21–50 years, 51 of whom men and 28 women. All subjects were regarded as otologically normal with no history of ear disease and a normal ear examination. The hearing threshold was within 0–20 dB (related to ISO standard, 1964). All subjects were free from signs of catarrhal infection at nose and throat examination and there was no history of labyrinthine disease.

## EXPERIMENTAL PROCEDURE

### A. Passive equilibration at simulated ascents—monaural recording

In this part of the present study recordings were made only from one ear in each examination as there was no equipment for binaural recording. The forcing pressure was defined as the level of the chamber pressure change at the moment of passive forcing. The middle ear pressure ( $P_m$ ) was not calculated.

(1) All subjects (79) were examined in the seated position and the  $V_{tm}$  was recorded during simulated ascents from +45 to -45 cm H<sub>2</sub>O in 25 sec. The subjects were instructed to avoid active equilibration of the middle ear pressure during each ascent.

(2) Re-examinations at intervals of several



months were made on 14 subjects (18 ears) in order to assess the individual variation of the forcing pressure from one time to another. The examinations were performed in the same way as described above.

## B Active and passive equilibration at simulated ascents and descents—binaural recordings and simultaneous ENG-recording of eye-movements

In this part of the present study recordings were made from both ears simultaneously. Ptm at the moment of passive forcing was calculated. Eight subjects were examined in the seated position and Vtm was recorded from both sides together with ENG. The subjects were instructed to keep their eyes open in total darkness, to look straight ahead and to avoid blinking. During the tests with active equilibration the subjects were told to swallow as often as possible and during the tests with passive equilibration they were instructed to avoid active regulation of the middle ear pressure.

## RESULTS

### A Monaural recording of passive equilibration at simulated ascents

1 The tube was forced open in all subjects when exposed to simulated ascents. The forcing of the tube was caused by overpressure in the middle ear due to the pressure decrease in the chamber.

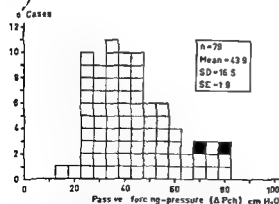


Fig. 4. Distribution of 79 otologically healthy subjects in relation to passive forcing pressure (abscissa). The forcing pressure is given as the pressure change in the chamber ( $\Delta Pch$ ) necessary to force the Eustachian tubes open. Black squares indicate two subjects who reported vertigo during the examination.

Table 1. Statistical evaluation of the individual variation of the passive forcing pressure recorded on 14 subjects (18 ears) at different sessions and with different numbers of examinations.

Ears	No. of sessions	No. of examinations	Passive forcing pressure ( $\Delta Pch$ ) cm H <sub>2</sub> O	
			$\bar{x}$	$d(\bar{x})$
1	4	9	67.2	2.6
2	2	4	43.3	3.8
3	2	4	69.8	3.8
4	3	14	75.1	2.7
5	2	14	49.6	3.3
6	2	11	82.3	3.4
7	3	15	71.3	2.8
8	2	13	51.1	3.3
9	3	17	68.1	2.7
10	2	5	31.3	3.7
11	2	5	49.6	3.5
12	3	9	46	2.8
13	5	21	36	2.3
14	4	9	46.3	2.6
15	3	7	39	2.9
16	4	14	77.9	2.5
17	2	12	47.4	3.3
18	6	26	31.8	2.0

The forcing occurred at different pressure levels in different subjects. The distribution of the subjects as regards the forcing of the tubes in relation to the pressure decrease in the chamber is given in Fig. 4. Two out of 79 subjects reported a brief period of vertigo during the examination and these 2 subjects are indicated in Fig. 4 by black squares. It was possible to reproduce the vertigo in these 2 subjects when re-examined months later, and simultaneous ENG revealed horizontal nystagmus indicating that the vertigo was of vestibular origin. Vertical nystagmus was never seen.

2 In Table 1 the results of the re-examination of 18 ears are presented. The passive forcing pressure is given as the ambient pressure change ( $\Delta Pch$ ) at the moment of passive forcing of the tubes. The Table presents a statistical evaluation of the results of the examinations at different sessions. The variation of the forcing pressure

each subject was low despite intervals of several months between the different sessions

*Comment* As seen from Fig 4, the tubes of the two subjects with vertigo were not forced open until there was a great pressure decrease in the chamber indicating that the forcing pressure in these subjects was high. As however the recordings were made only from one side, it has so far been impossible to find out from which ear the vertigo was elicited. These two subjects were examined with pressure recordings from both sides simultaneously together with ENG.

*Binaural recording of passive and active equilibration during simulated ascents and descents*

The two subjects reported vertigo each time when exposed to ascents and passive clearing of the ears. Horizontal nystagmus coincided with every period of vertigo. In each subject the left and the right tube were forced open at different chamber pressure levels, indicating different forcing pressure for the left and the right tube.

In Table II is presented the magnitude of the pressure decrease in the chamber ( $\Delta Pch$ ) at which the tubes were forced open, recorded at different examinations. (The Table also includes the monaural recording of these two subjects.) Nystagmus intensity, expressed as the maximal velocity of the slow nystagmus phase and reports of vertigo are also indicated. No nystagmus during ascents with active equilibration was seen and no vertigo was reported. Descents with and without active equilibration caused neither vertigo nor nystagmus, and there was no passive clearing when the subjects were exposed to descents.

Both subjects could equilibrate static as well as dynamic over and under pressures in a normal way, and belonged to tubal function groups IB and IC (very good tubal function) according to the tubal function groups presented by Elnér et al (1971b).

Fig 5 shows a recording at a simulated ascent with passive clearing of the ears. The left tube was forced open when the ambient pressure was lowered by 49.5 cm H<sub>2</sub>O (a), and the right tube

**Table II** Chamber pressure decrease ( $\Delta Pch$ ) at which the Eustachian tubes were forced open as recorded at different sessions with different numbers of examinations

The Table also includes the monaural recordings of the two vertiginous subjects. Nystagmus intensity and reports of vertigo as indicated

Session no	Passive forcing cm H <sub>2</sub> O		Vertigo	Max. velocity of eye-deviation in slow phase, °/sec
	$\Delta Pch_{dx}$	$\Delta Pch_{sis}$		
<i>Subject I</i>				
1	81	Not recorded	+	Not recorded
2	79.5	Not recorded	+	3
	82.5	Not recorded	+	3
3	81	49.5	+	4.5
	87	48	+	3
	78	48	+	5
	79.5	46.5	+	5
	81	46.5	+	3.5
<i>Subject II</i>				
1	72.5	Not recorded	+	Not recorded
2	67.5	Not recorded	+	Not recorded
	76.5	Not recorded	+	2
	72	Not recorded	+	3.5
	64.5	46.5	+	1
3	69	39	+	1
4	60.5	43.5	-	0.5
	64.5	43.5	-	2.5

when lowered by about 81 cm H<sub>2</sub>O (b). When the ambient pressure was lowered by about 66 cm H<sub>2</sub>O an eye-deviation started, directed to the left (c), and was followed by nystagmus to the right. The right beating nystagmus continued until the right tube was forced open, after which it decreased and changed direction, and a left-beating nystagmus was recorded for about 20-30 sec. Just before the right tube was forced open the subject reported vertigo.

*Comment* A very interesting feature of this recording is that the vestibular stimulation was not induced at the moment of pressure regula-

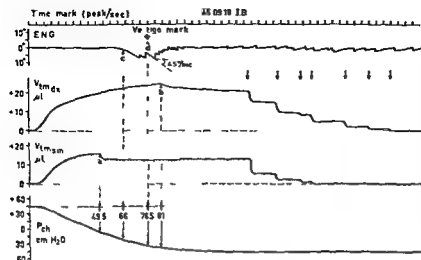


Fig 5 Electronystagmogram (ENG) volume displacements of left ( $V_{tmL}$ ) and right ( $V_{tmR}$ ) ear drums and chamber pressure ( $P_{ch}$  in  $\text{cm H}_2\text{O}$ ) during passive forcing of the Eustachian tube. Arrows mark deglutitions. Passive clearing of left ear (a) and right ear (b). Nystagmus starts when ambient pressure is changed by  $\text{cm H}_2\text{O}$  (c)—slow nystagmus plot. The subject reports vertigo when the chamber pressure lowered by  $76.5 \text{ cm H}_2\text{O}$  (further comments see text).

tion, but during the period of asymmetric middle ear pressure and not until the pressure in the not-cleared ear had reached a certain level. To assess the asymmetry and to find out the real difference between the middle ear pressure and the ambient pressure, i.e. the pressure gradient across the eardrum ( $P_{tm}$ ) it was necessary to calculate the middle ear pressure on both sides at the moment of passive forcing (see eqs 1 and 2).

$\Delta P_{ch}$  and  $\Delta V_{tm}$  were calculated directly from the recordings. The middle ear volumes were determined by the planimeter method and transformed from area ( $A_m$ ) to volume ( $V_m$ ) by using correction factors determined by Andréasson (1973). The results are given in Table III.

In the material presented by Andréasson (1973) there were no normal subjects but only subjects with a traumatic perforation of the eardrum. As however the figures of the area and the volume of these subjects fall within the limits of normal values as described by other authors (Diamant, 1940; Ingelstedt et al, 1967; Elnér,

1971), it was assumed that the figures are valid also for the normal subjects in the present study. In order to verify this assumption the middle ear volume on one side of 2 subjects (subject I and IV) was determined both indirectly according to Ingelstedt et al (1967) and Elnér et

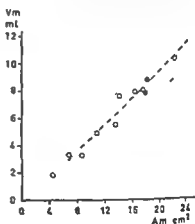


Fig 6 Comparison between area ( $A_m$ ) in  $\text{cm}^2$  and volume ( $V_m$ ) in ml (Andréasson 1973).  $\circ$ —9 subjects with traumatic perforation of the eardrum. The area determined by a roentgenological planimetric method (Diamant 1940; Flisberg & Zsmond 1965; Andréasson 1973) and the volume by a direct volume determination of the air-filled middle ear system (Flisberg & Zsmond 1965; Andréasson 1973).  $\bullet$ —2 subjects with a normal ear examination. The area was determined with the roentgenological planimetric method as mentioned above and the volume by an indirect determination according to Ingelstedt et al (1967) and Elnér et al (1971a). Determinations were made from the right side of both subjects. The lines of short dashes (curves) indicate 95% confidence belt for a predicted 'y' (Snedecor & Cochran 1973a).

Table III Area in  $\text{cm}^2$  and volume in ml of the middle ear cell system

Subject	$\Delta V_{tm}$		$\Delta P_{ch}$	
	$A_m$	$V_m$	$A_m$	$V_m$
I	21.4	10.9	19.4	9.1
II	15.3	7.1	13.2	6.1

(1971a) and by the planimeter method, and it was seen that these two different volume determinations gave very similar results (Fig 6) The results of the calculation of the pressure gradient across the eardrum (Ptm) at the moment of passive forcing of the tubes are given in Table IV

*Comment* As seen from Fig 4, there were however another 7 subjects with a high forcing pressure ( $\Delta Pch > 65$  cm H<sub>2</sub>O), none of whom had complained of vertigo. Out of these 7 subjects 4 were re-examined with binaural recordings and ENG, and 2 subjects with a forcing pressure according to the mean value ('normal subjects') were also included

Three out of 4 subjects with high forcing pressures reported vertigo at one occasion each when re-examined (subject VI, VII and VIII) Asymmetric forcing pressures were also seen in

Table IV Chamber pressure decrease ( $\Delta Pch$ ) and pressure gradient across the eardrum (Ptm) at the moment of passive opening of the Eustachian tubes recorded at different sessions and with different numbers of examinations

The Table also includes the monaural recordings of the two vertiginous subjects

Session no	Passive forcing cm H <sub>2</sub> O			
	$\Delta Pch_{ax}$	Ptm <sub>ax</sub>	$\Delta Pch_{sin}$	Ptm <sub>sin</sub>
<i>Subject I</i>				
1	81	75.9	Not recorded	
2	79.5	74.4	Not recorded	
	82.5	77.4		
3	81	75.4	49.5	45.4
	87	81.1	48	44
	78	72.6	48	44
	79.5	74	46.5	42.5
	III	75.4	46.5	42.5
<i>Subject II</i>				
1	72.5	65.7	Not recorded	
	67.5	61		
2	76.5	68.5	Not recorded	
	72	64.9		
3	64.5	58.2	46.5	40.7
	69	62.4	39	33.9
	60.5	54.6	43.5	38.2
4	64.5	57.7	43.5	38.2

Table V. Area in cm<sup>2</sup> and volume in ml of the middle ear cell system

Subject	Dx		Sin	
	Am	Vm	Am	Vm
III	18.3	8.6	22.2	10.6
IV	17.8	8.3	17.8	8.3
V	23.8	11.2	25.8	12.3
VI	21.5	10.5	22.9	10.8
VII	25.5	12.4	23.8	11.6
VIII	17.0	7.8	17.3	8.1

these subjects Horizontal nystagmus was recorded at every test in which vertigo was reported. However, nystagmus was also seen in some of the tests though the subjects did not notice any vestibular stimulation. When the 2 subjects with a normal forcing pressure were re-examined no nystagmus was seen and no one reported vertigo. The middle ear volumes were calculated by the planimeter method and transferred from area (Am) to volume (Vm). The results of these calculations are given in Table V.

The results of the calculation of Ptm at the moment of passive forcing of the tubes are given in Table VI. The table also includes the monaural recordings of the subjects.

During ascents with active equilibration no nystagmus was seen and no vertigo was reported. Descents with and without active equilibration caused neither vertigo nor nystagmus in any subject, nor was there any passive opening of the tube when the subjects were exposed to descents. All subjects could equilibrate static as well as dynamic over- and underpressures in a normal way and all belonged to tubal function groups Ib and Ic according to Elner et al (1971b).

It was also of interest to calculate the pressure gradient across the eardrum (Ptm) and the pressure difference between the ears at the moment when nystagmus started (Nystagmus pressure level-NPL). Table VII presents the NPL for each ear in the subjects with nystagmus during simulated ascents.

*Comment* As seen from these results there is only a small variation in the pressure level at

Table VI Pressure gradient across the eardrum (Ptm) at the moment of passive opening of the Eustachian tubes recorded at different sessions and with different numbers of examinations

Subject	Session no	Passive forcing cm H <sub>2</sub> O					
		Ptm <sub>ex</sub>			Ptm <sub>sin</sub>		
		n	Mean	Range	n	Mean	Range
III	1	3	21.7	21.4-22.0	10	16.3	15.6-18.6
	2	7	25.8	24.6-27.8			
	3	2	29.3	28.0-30.6			
	4	1	29.0	29.0			
	5	3	33.3	31.7-34.6			
	6	10	28.2	24.9-34.6			
IV	1	6	32.7	30.0-34.1	5	38.9	35.3-42.3
	2	2	32.6	32.5-32.6			
	3	2	34.6	33.9-35.3			
	4	1	29.7	29.7			
	5	10	33.5	28.9-36.8			
V	1	2	76.2	75.5-76.9	5	50.9	46.0-56.0
	2	5	63.0	61.3-65.5			
	3	8	59.6	51.0-65.6			
VI	1	3	71.8	68.5-74.1	8	47.6	43.1-50.2
	2	6	70.6	65.4-74.1			
	3	8	67.2	63.8-70.9			
VII	1	1	70.9	70.9	3	66.1	65.2-67.9
	2	3	60.8	59.4-62.2			
VIII	1	2	79.4	76.6-82.2	9	47.6	38.6-54.4
	2	9	70.4	68.1-73.4			

which nystagmus starts within each subject, recorded at different tests. The magnitude of the asymmetry did not seem to influence the degree of vestibular stimulation in subject II. The great asymmetry in this subject is not caused by any variation of the forcing pressure on the left hand side, but by the fact that the left ear was not cleared before the ascent (test 2, third examination). The subject had a relative underpressure in that ear at the beginning of the ascent.

## DISCUSSION

The present study shows that it is possible to elicit A/V in a pressure chamber in certain subjects when exposed to simulated ascents with passive clearing of the ears. The experimental situation is close to conditions that may be encountered in actual flying and diving, as most pilots and divers usually do not clear their ears actively during ascents. Some authors are however of the opinion that even an ambient pres-

sure increase (descent) might cause vertigo (Allstrong & Heim, 1937; Benson, 1965; DeWeese & Saunders, 1960). This condition, however, could not be reproduced in the present study despite of a pressure increase of about 90 cm H<sub>2</sub>O in 25 sec, which in several subjects caused a relative underpressure in the middle ears of about the same magnitude. It also appears from the present study that it was possible to elicit A/V in 5 out of 79 subjects all regarded as normal. All of these 5 subjects had a high forcing pressure on one side when compared with the mean value of the whole material. Repeated examinations with intervals of several months reveal that the high forcing pressures could be reproduced and the individual variation of the forcing pressures was low. Asymmetry between the left and the right ear was also seen. Asymmetry between the ears with regard to the ease with which pressure equilibration takes place has earlier been recognized in interview studies of vertigo-prone divers and flyers (Lundgren, 1965; Luv-

Table VII Chamber pressure decrease ( $\Delta Pch$ ), pressure gradient across the eardrum ( $Ptm$ ) and the pressure difference between the ears at the moment the start of nystagmus was recorded ( $N$ ), nystagmus pressure level - NPL)

go incidents and nystagmus intensity are also given. Nystagmus was directed to the right in all examinations those indicated by +

Subject	Session	Nystagmus pressure level - NPL (cm H <sub>2</sub> O)				Max. velocity of eye-deviation in slow phase (°/sec)	Vertigo
		$\Delta Pch$	$Ptm_{ax}$	$Ptm_{min}$	$Ptm_{ax} - Ptm_{min}$		
I	2	69	64.4	Not recorded		3	+
		73.5	68.7	Not recorded		3	+
	3	66	61.3	16	45.3	4.5	+
		64.5	59.8	17.3	42.5	3	+
		66	61.3	15.5	45.8	5	+
		66	61.3	16.1	45.2	5	+
		69	64.1	16.9	47.2	3.5	+
II	2	66	59.3	Not recorded		2	+
		67.5	60.7	Not recorded		3.5	+
	3	57	51.2	32.3	18.9	1	+
		64.5	58.2	14.5	43.7	1	+
		60	54	22.7	31.3	0.5	+
	4	60	54	24	30	2.5	+
VI	2	66	61.1	12	49.1	2	+
VII	2	64.5	40.5	60.7	20.2	3.5*	+
		70.5	40.5	66.4	-25.9	2*	-
VIII	2	66	61	16.5	44.5	0.5	-
		72	66.5	21	45.5	0.5	-
		67.5	62.3	16.5	45.8	1.5	+

ren & Malm 1966). In the present study, however, it was difficult to estimate the importance of the degree of the asymmetry for eliciting vertigo. As is seen from the results (subject II), roughly the same vestibular stimulation was recorded during two different tests though there was a great variation in asymmetry between the ears (examination 1 and 2 in session 3, cf. Table VII). It is also seen from the tests with subject VII that despite a much smaller asymmetry, as compared with that of the other subjects, inner ear stimulation was recorded.

The mechanism for A V is, however, still unknown. It has been suggested (Melvill Jones, 1957; Benson, 1965), that the vertigo might be induced by sudden movements of the stapes due to a sudden pressure change in the middle ear following regulation or Valsalva's manoeuvre.

It is difficult, however, to connect this brief stimulation with the duration of the vertigo, which by some subjects is reported to last for 15 minutes (Lundgren, 1965; Vorosmarti & Bradley, 1970). It appears from Fig. 5, however, that the vestibular stimulation is not induced at the moment of pressure regulation as an effect of sudden movements of the stapes, but during the period of asymmetric middle ear pressure and not until the relative overpressure in the "not-cleared" ear has reached a certain level. This observation means that there might be another explanation of the way in which the pressure acts upon the inner ear than that previously suggested, or possibly that A V might be elicited in more than one way. If the vestibular stimulation is caused by a cupula deviation it has to be by way of a fluid movement in the laby-

rinth. Anatomically it is reasonable to expect that large displacements of the stapes footplate might cause a fluid flow in the inner ear. However, when overpressure in the middle ear is considered as the cause of vertigo, it should be realized that the pressure acts equally on the two windows. Then the labyrinthine fluid would not be set in motion unless there is a place for the fluid to escape. Fluid displacement through the perilymphatic and the endolymphatic ducts might be considered. However, the function of these structures is not known exactly. Overpressure in the middle ear might also move the stapes footplate against the pressure gradient as the ossicles are mechanically linked to the eardrum and simultaneously push the membrane, covering the round window, so that it bulges into the inner ear, in this way setting the inner ear fluid in motion. The function of the ossicular chain in such a case is however not exactly known, as the joints between the ossicles might "slip" when the movements of the eardrum are of large amplitude.

As for the mechanism through which overpressure in the middle ear may induce vertigo and nystagmus, a circulatory insufficiency in the inner ear caused by overpressure is also suggested. Blood vessel connections between the middle ear and the inner ear structures have been demonstrated (e.g. Hansen, 1971), and as the pressure level is recorded to be about 60 cm H<sub>2</sub>O in the case of vestibular symptoms, there is a possibility of a hypoxaemia due to venous stasis and/or arterial insufficiency.

Another observation in the present study was that the subjects with a high forcing pressure on the right hand side got nystagmus to the right, and one subject (subject VII) with the highest pressure in the left ear got nystagmus to the left. It should also be observed that the vestibularly induced eyeball deviation starts before the subject observes the stimulation and that nystagmus was recorded in some tests although the subjects did not observe vestibular stimulation.

It is seen from the present study that certain subjects, although otherwise otologically normal, are more susceptible to experience of A V

because of an inclination to a permanent high forcing pressure on one side. A normal forcing pressure might however be influenced and increased by different factors such as catarrhal infections, other inflammatory conditions, oedema due to repeated barotrauma or venous stasis of different origin for example caused by different positions of the body (Ingelstedt et al 1967, Rundcrantz, 1969). It is seen from our reports that a great many subjects with experience of A V have also reported common vertigo in connection with these symptoms (Mel-Jones, 1957, Benson, 1965, Lundgren, 1969, Vorosmarti & Bradley, 1969, Brown, 1971).

The method presented above might be useful for improving flying and diving safety by eliminating subjects susceptible to vertigo caused by middle ear mechanics as described in this paper. It appears that it is not possible to make such a selection by using "ordinary" tubal function tests only, but that the passive equilibration capacity must be tested. The capacity of passive forcing is not a tubal function in a physiological sense and should not be confused with such function, since no active muscle work is involved in opening the tube. There is no correlation between the active and passive equilibration as is seen in the present study: the subjects with a high forcing pressure could all equilibrate in a normal way. When using the method presented above in routine examinations of otologically normal subjects, it seems sufficient to determine the ambient pressure change ( $\Delta P_{ch}$ ) needed to force the Eustachian tubes open, as there will be only small differences between  $P_{tm}$  and  $\Delta P$  as long as the middle ear volume ( $V_m$ ) is not too small ( $< 6$  ml, see equation 1 and 2). This means that the area of the air-filled middle ear cavity should not be less than about 13 cm<sup>2</sup>. It further seems possible to prevent A V by frequent equilibration during ascents also, since no nystagmus was recorded in tests with vertigo-prone subjects at ascents with active equilibration.

#### Statistical Comment

In the evaluation of the individual variations of the passive forcing pressure (cf. Table I) a mean

with two variance components was assumed; for variance "between sessions" and  $\sigma^2$  for variance "between examinations within sessions". The variance components were estimated jointly from all cases. The estimates  $\sigma_s^2 = 19.5$  and  $\sigma_e^2 = 13.0$  were obtained. The corresponding standard error  $\sigma_s = 4.4$  and  $\sigma_e = 3.6$ .

An analysis of variance for each case was performed, i.e. the sums of squares "between sessions" and "between examinations within sessions" were calculated. The estimates of  $\sigma_s^2$  and  $\sigma_e^2$  were based on the sum of the sums of squares "between sessions" for all cases and the sum of the sums of squares "between examinations within sessions" for all cases (Snedecor & Cochran, 1973b).

## ZUSAMMENFASSUNG

Bei 5 von 79 otologisch gesunden Versuchspersonen konnte durch simulierten Aufstieg und passiven Druckausgleich Schwindel hervorgerufen werden. Die experimentelle Situation glich sehr der Wirklichkeit, da die meisten Piloten und Taucher während des Aufstiegs aktiven Druckausgleich vornehmen, sondern ihren passiven Ausgleich abwarten, der eintritt, wenn die des relativen Überdrucks im Mittelohr gross genug ist, um die Eustachische Tube zwangsmässig zu öffnen. Bei den Versuchspersonen, die Schwindel angaben, verglichen mit dem Mittelwert von 79 normalen Versuchspersonen einseitig ein erhöhter Öffnungsdruck. Die Tube festgestellt werden. Während der Dauer des Schwindels wurde regelmässig ein gleichseitiger Nystagmus festgestellt. Diese vestibuläre Stimulation wurde nicht im Augenblick des Druckausgleiches und somit auch die Wirkung schneller Stapesbewegungen ausgelöst. Vielmehr erfolgte der Nystagmus während der gesamten entspannten asymmetrischen Mittelohrdrucks, aber erst nachdem der relative Überdruck im „nichtausgeglichenen“ Ohr ein gewisses Mindestmass erreicht hatte.

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## VESTIBULAR SYMPTOMS IN IDIOPATHIC FACIAL PALSY

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(Received December 28, 1973)

**Abstract** One week after the onset of the paralysis 75%, respectively 92% of the patients with Bell's palsy presented with a spontaneous or/and positional nystagmus. The spontaneous vestibular symptoms are discrete and usually not subjectively realized by the patients. No direct correlation between the degree of the vestibular disturbance and the severity of the facial nerve palsy has been found in the first week after onset of the paralysis. Only the time evolution of the spontaneous vestibular signs (particularly of the positional nystagmus) showed whether the lesion of the VII nerve was reversible or not. The present study does not confirm therefore the hypothesis that the vestibular signs present in Bell's palsy may be used for the prognostic assessment of the lesion. The quality and evolution of the vestibular signs found in Bell's palsy confirm, on the other hand, the surgical observation that the most common site of lesion of the facial nerve in idiopathic palsy is the entrance of the Fallopian Canal and not in its more distal course.

Vestibular symptoms occur quite frequently in the presence of idiopathic facial palsy (Philipszoon, 1962; Robert & Pfaltz, 1970). Their origin is most probably related to the compression of the superior branch of the vestibular nerve by the edematous facial nerve fibres in the region of the meatal fundus (Fisch & Esslen, 1972; Fisch, 1973). The aim of the present study was to determine the pattern and time evolution of the vestibular disturbances observed in Bell's palsy and to analyse whether the vestibular symptoms correlate with the degree of severity of the palsy.

### MATERIAL AND METHODS

The spontaneous and positional nystagmus as well as the caloric reaction of 25 patients (average age 33 years) have been recorded by electro-

nystagmography at regular intervals for 6 months after the onset of a unilateral idiopathic facial paralysis. The caloric test was performed by irrigating the external ear canal with 20 cc of water of 27 or 47°C. The following parameters of the caloric nystagmus were analysed: duration, average maximal velocity of the slow component, frequency, and total maximal intensity. In order to correlate the electronystagmographic findings with the degree of the palsy, the percentage of degenerated facial nerve fibres was determined by recording the summing potentials of the paralysed muscles of the face according to a method recently described by Esslen (1973).  $\square$

### RESULTS

The evolution of the spontaneous vestibular signs in 25 patients with idiopathic facial palsy are shown in Fig. 1. One week after the onset of the paralysis 75%, respectively 92% of the patients evidenced a spontaneous nystagmus or/and respectively a positional nystagmus. The average velocity of the slow component of the spontaneous nystagmus was, on average, 2-3°/sec, its maximal velocity 5° per sec. The number of patients with spontaneous vestibular signs decreasing rapidly in the first 4 weeks and slowly thereafter. The percentage of patients with spontaneous nystagmus reached normal average values (16% of the cases) 4 months after onset of the palsy. The positional nystagmus was still present in 40% of the patients at the end of the observation of 6 months. The direction of the

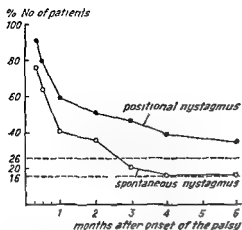


Fig 1 Time evolution of the spontaneous and positional nystagmus in 25 patients with idiopathic facial paralysis. The broken lines represent the percentage of spontaneous (16%) and positional (26%) nystagmus found in a group of normal individuals with the same age as the investigated population.

spontaneous vestibular signs did not change during the recovery from the palsy, pointing towards the healthy side in 2/3 and towards the affected side in 1/3 of the cases. 20% of the patients developed a direction changing positional nystagmus shortly before the disappearance of their spontaneous nystagmus.

The analysis of the caloric response gave less

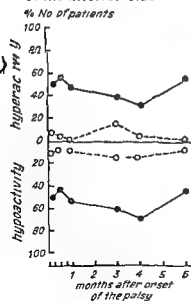
clear results than that of the spontaneous vestibular signs. The ear of the affected side was hypoactive in one-half and hyperactive in the other half of the cases (Fig 2). Later on, the prevailing hyperactivity (second week) is followed by a definite trend towards hypoactivity (third and fourth month). A similar pattern is shown by the few cases (less than 20%) having a significantly reduced or increased caloric response.<sup>1</sup> The directional preponderance (Fig 2B) is mostly oriented towards the side of the palsy, particularly during the first 2 months after onset of the palsy.

Fig 3 shows the spontaneous vestibular signs in relation to the degree of the facial paralysis. Four groups of patients have been formed according to the results of the neuro-electric tests:

- (1) patients with axon-blockade only
- (2) patients with less than 50% degenerated facial nerve fibres

<sup>1</sup> According to the normal distribution of the caloric nystagmus the unilateral response is significantly reduced or increased if values of more, respectively less than 25 are obtained by the formula  $(1+3)-(2+4)/(1+3+2) \times 100\%$ .

#### A Hypo- or hyperactivity of the involved side



#### B Directional preponderance

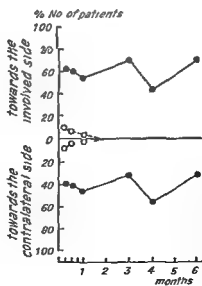


Fig 2 Time evolution of intensity of the caloric nystagmus (maximal velocity in the slow phase) in 25 patients with idiopathic facial paralysis. Note that only a limited group of patients with Bell's palsy present with significantly disturbed caloric response.

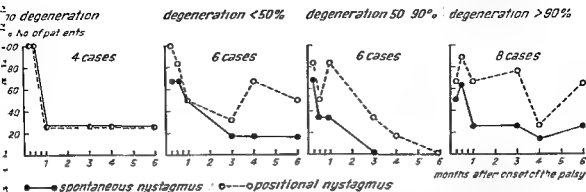


Fig. 3 Relation between spontaneous and positional nystagmus and the degree of degeneration of the facial nerve in 24 patients with idiopathic palsy. Note that there is no direct correlation between the degree of vestibular symptoms and the severity of the facial nerve degeneration during the first week after onset of the paralysis.

The later time evolution of the spontaneous and particularly of the positional nystagmus are however delayed, particularly if more than 90% of the facial nerve fibres are degenerated.

- 3) patients with 50 to 90% degenerated facial nerve fibres
- 4) patients with total degeneration of the facial nerve

The spontaneous vestibular signs disappeared more rapidly (4 weeks) in presence of an axon-blockade only than in patients with partial or total facial nerve degeneration.

This observation indicated that in Bell's palsy the time evolution of the spontaneous vestibular signs reflects the degree of lesion of the nerve fibres.

The correlation between the caloric nystagmus

and the degree of the facial nerve lesion showed that the axon blockade is rather followed by the hypoactivity whereas the degeneration of the nerve fibres is followed by hyperactivity of the vestibular response on the affected side.

The total maximal intensity of the caloric response (Fig. 4) remained well above normal average values during the first three months of the palsy.

## DISCUSSION

75%, respectively 92% of the investigated patients with idiopathic facial palsy presented with a spontaneous or/and positional nystagmus one week after onset of the paralysis (Fig. 1). The direction of the spontaneous vestibular signs (towards the unaffected side) indicates that a rather severe lesion of the utricle-ampullar nerve most probably due to compression from the edematous facial nerve (Fisch & Esslen 1972) takes place in 2/3 of the patients suffering from Bell's palsy. The recovery from the vestibular disturbance is very rapid during the first 2 weeks after onset of the palsy and slows down thereafter. The time-evolution of the spontaneous vestibular signs reflects therefore the acute phase of Bell's palsy, which is determinant for the destiny of the facial nerve fibres and rarely extends beyond the first 10-12 days after onset of the palsy (Esslen & Fisch, 1971).

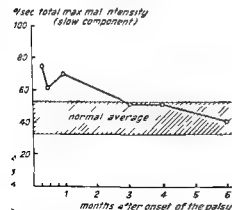


Fig. 4 Evolution of the total maximal intensity of the caloric reaction of 25 patients with idiopathic facial palsy. Note the increased maximal intensity for as long as 3 months after onset of the paralysis.

The correlation between presence, respectively evolution of spontaneous vestibular signs and the degree of degeneration of the facial nerve fibres shows (Fig 3)

(1) that the spontaneous vestibular signs are more frequently observed in patients without than in patients with degeneration of the VII nerve, and

(2) that spontaneous and positional nystagmus disappeared more rapidly if the facial nerve fibres did not degenerate

According to these observations it is not the presence but the time-evolution of the spontaneous vestibular signs which correlates with the severity of the facial palsy. This is to be expected since spontaneous and positional nystagmus can result from a reversible axon-blockade or from an irreversible degeneration of the afferent vestibular nerve fibres. Only the time of recovery will permit a differentiation between these two different neural conditions. It is also logical to suppose that the less important lesion producing a reversible blockade for impulse conduction in the facial nerve will also be followed by reversible changes in the fibres of the utriculo-ampullar division of the vestibular nerve.

In view of the discrete character of the registered spontaneous vestibular signs it is not surprising that the caloric reaction of patients with Bell's palsy is only insignificantly disturbed. This may also result from the fact that the intracranial compression from the edematous facial nerve involves particularly the utricular nerve, which is closer to it and to a lesser extent the laterally situated superior and horizontal ampullar fibres.

In conclusion, there is no direct correlation between the degree of the vestibular disturbance and the severity of the facial nerve palsy during the first week after onset of the paralysis. Only the later time-evolution of the spontaneous vestibular signs (particularly of the positional nystagmus) shows whether the lesion of the VII nerve was reversible or not. The present study does not confirm, therefore, the hypothesis that the vestibular signs present in Bell's palsy may be used for the prognostic assessment of the

lesion. The quality and evolution of the vestibular signs found in Bell's palsy do confirm the other hand, the surgical observation that the most common site of lesion of the facial nerve in idiopathic palsy is the entrance of the nerve into the internal acoustic meatus (the vestibular Canal) and not its more distal course.

## ACKNOWLEDGEMENT

We acknowledge the technical assistance of Mrs Regeler.

## ZUSAMMENFASSUNG

Ein Spontan- oder Lagennystagmus wurde bei 75% bzw. 92% von 25 Patienten mit einer einseitigen

Störung und Schwere der Paresis konnte in der Woche nach Einsetzen der Läsion nicht nachgewiesen werden. Der weitere Verlauf der vestibulären Störung (vor allem der Lagennystagmus) zeigte dagegen, dass die Läsion des Gesichtsnerven reversibel war oder nicht. Vorliegende Untersuchung zeigt, dass aus der vestibulären Symptomatik kleine Schlüsse in bezug auf die Prognose einer Bell'schen Paresis gezogen werden können. Die zeitliche Entwicklung der vestibulären Zeichen bei einer idiopathischen Paresis bestätigen, dass der Ort der Läsion häufigsten am Anfang des Fallopiischen Kanals und weiter distal liegen muss.

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## POSITIONAL AND POSITIONING NYSTAGMUS AS A RESULT OF UTRICULOCUPULAR INTEGRATION

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(Received December 19, 1973)

**Abstract** A short review of the positional nystagmus problem is given. The different planes of position for obtaining positional nystagmus are described and its different types discussed. The results of experiments during recent years on animals and on man are presented and clinical conclusions are drawn from these results. Positional and positioning nystagmus are defined, and the most suitable positions for positional nystagmus are mentioned. Normal and pathological results which are to be expected from the labyrinth during these positions are presented. A method of studying positioning horizontal nystagmus as a result of integrated utriculocupular activity is described and the results discussed.

Positional nystagmus is generally defined as a spontaneous nystagmus which is not constantly present, but appears only in certain positions (Barany, 1913, Voss, 1921, Bornes, 1923, Gerlings, 1948, Nylen, 1950). To this definition Aubry et al (1954) want to add one more group with spontaneous nystagmus in normal positions, which changes appearance or intensity in a certain position of the body.

As early as 1913 this phenomenon was described by Barany, who in 1924 was able to show that these reactions were released by position and not by movement. The problem has subsequently been elucidated by hundreds of authors, who stated that positional nystagmus is observed in diseases of many different kinds both of the peripheral labyrinth and of the central nervous system (See Frenzel, 1961a).

### *Planes of position*

The method used for investigating positional nystagmus varies from author to author, but

principally the investigation is made in two different planes (Nylen, 1950, Aubry et al, 1954).

1 The sagittal plane in a sitting or supine position, or with the head hanging out over the upper edge of the investigation table.

2 The frontal plane, in a sitting position with the head tilted over one shoulder, or in a right or a left lateral supine position.

Nylen recommends that the patient's head and body should move together to exclude the so-called neck reflexes and that change of position should take place very slowly. Usually, the position should be changed 90° in 5 sec. However, for scientific purposes he recommends a slow constant speed of 1-2° per sec without any acceleration. Dix & Hallpike (1952), Cawthorne (1954) and Cawthorne & Hallpike (1957) recommend that from a sitting position the patient should rapidly be brought, within 3 sec, to a supine position with the head turned to either side. The head is moved independently of the body. Cawthorne considers that this has no importance for the results.

### *Types of positional nystagmus*

By using the above mentioned methods of investigation positional nystagmus has been studied in a great many diseases both of the peripheral labyrinth and of the central nervous system. It has been shown that positional nystagmus can be classified according to different types. Nylen has been one of the pioneers in this field. Between 1924 and 1950 he published

several papers on positional nystagmus. He proposed the following classification:

*Type 1* Direction-changing, positional nystagmus, which is characterized by a nystagmus changing direction in different head positions. Consequently, in one position nystagmus can be horizontal, and in the opposite position vertical or rotatory.

*Type 2* Direction fixed, positional nystagmus, where nystagmus is invariably in the same direction, when the subject is in different positions. If nystagmus is observed in all head positions, it is definitely altered in intensity in a certain position.

*Type 3* Irregular, positional nystagmus, characterized by variations in its behaviour.

Other authors have made similar classifications, which, however, except for minor variations, coincide with the above mentioned classification (Ruttin, 1936; Seiferth, 1937; Frenzel, 1938; Lindsay, 1951; Aubry et al., 1954).

Most scientists seem to agree that positional nystagmus Type 2 usually occurs in peripheral labyrinthine diseases (Blomqvist, 1948; Gerlings, 1948; Nylen, 1950; Cawthorne, 1954; Aubry et al., 1954; Lindsay, 1951), even though this type also occurs in central diseases (Fromm, 1934; Nylen, 1939, 1950). Type 1 and Type 3 are mostly observed in central diseases of different kinds.

However, the different authors have not only described the type of positional nystagmus they found but also its appearance and duration. Barany described a case with horizontal nystagmus to the left in a right lateral position. In 1921 he published a report on another case with rotatory nystagmus with the upper pole of the eyes beating to the right in a right lateral position. Voss (1921) has observed horizontal nystagmus to the left, when the patient leaned his head over his right shoulder. Ruttin (1936) and Vogel (1950) described several labyrinthine injuries, with rotatory, positional nystagmus in the same direction as the patient is tilted. The duration was only a few seconds. Nylen (1950) observed a rotatory nystagmus in one lateral position and a horizontal nystagmus in the other

lateral position. The nystagmus was usually considered to be tonic, i.e., inexhaustible. Sometimes he found that the duration of nystagmus was only a few seconds. Dix & Hallpike (1952) and Cawthorne & Hallpike (1957) described rotatory or horizontal rotatory nystagmus beating towards the undermost ear with a duration not exceeding 10 sec. Mieb (1952) made the same observations and found that the duration was about 15 sec. Aubry (1954) found that positional nystagmus continued for as long as the patient held a certain position, but sometimes the durations were 30–40 sec. This problem has been specially studied by Stenger (1955), who distinguishes between "Lagenystagmus" and "Lagerungsnystagmus", i.e., nystagmus in a certain position and nystagmus immediately after positioning the patient in a certain position. He observed rotatory or horizontal-rotatory nystagmus. He considers that, as a rule, positional nystagmus is inexhaustible, whereas positioning nystagmus has a duration of only a few seconds.

It is evident that all these authors have observed either a horizontal or a rotatory nystagmus, or a combination of both. In those cases where the duration is mentioned, this is generally so short, that one must question whether the nystagmus was not due to the interference of angular acceleration. The same opinion has been expressed by Frenzel (1961b) and J. A. J. Kees (1961).

*The peripherally induced positional nystagmus*  
Even if the cause of positional nystagmus is to be found peripherally as well as centrally it is established that a functioning labyrinth at least on one side, is a prerequisite for elicitation of spontaneous as well as positional nystagmus. It is known that bilateral elimination of labyrinthine function extinguishes both these reactions (Spiegel & Scala, 1942; Dow, 1938; Allis & Fernandez, 1960). Rotatory eye movements are absent in bilaterally labyrinthectomized patients (Miller & Graybiel, 1963). Equivocal alcohol nystagmus does not appear in patients without labyrinthine function even though it is

pite of this, they are capable of becoming drunk (Aschan, 1961). Since the vestibular apparatus is a differential organ (Fluur & Mellström, 1971) it is the difference in input from the right and left labyrinth, which causes the ocular reactions, one has good reasons to assume that positional nystagmus of peripheral as well as central origin depends on a disturbance of the balance between the activity of the right and left vestibulo-ocular reflex arcs, and that the trigger mechanism for spontaneous as well as positional nystagmus is localized to the peripheral labyrinth. Therefore the present author has been interested in positional and positioning nystagmus, induced by interaction of input from the semicircular canals and otolith organs by tilting and which, so to say, give reactions according to the rules for a peripherally induced alteration of activity, whether this is normal or pathological.

As early as 1921 Bárány thought that positional nystagmus was a disorder of the otolith apparatus. The same opinion was held by Voss (1921), and later also by Chlrow (1927), Hasegawa (1939), de Kleyn & Versteegh (1930) and Dix & Hallpike (1952). Dix & Hallpike (1952) and Cawthorne & Hallpike (1957) have described histological findings, which favor the belief that the damage is localized in the utricle. Later, Schuknecht (1969) demonstrated histologically that positional nystagmus can also arise by substances of high specific gravity, possibly ototoxic, acting upon the cupula of the posterior semicircular canal.

Earlier the utricular effect on oculomotion was not known in detail, and furthermore, it was not known if the otolith organs really are capable of inducing nystagmus. However, investigations made during recent years, have thrown new light on this problem. Fluur & Mellström (1970) have shown that electrical stimulation of different areas on the utricular surface cause distinct eye movements. Subsequently Fluur & Siegborn (1973) in cat and Fluur (1973) in man, have demonstrated that these different areas co-operate with the semicircular canals in an intimate and lawful way in their influence on

the oculomotor system. The results can be summed up as follows. After a unilateral labyrinthectomy, horizontal nystagmus is facilitated if the patient is tilted around the longitudinal axis towards the diseased ear, and is inhibited if he is tilted towards the sound ear. After selective sectioning of the two anterior ampullar nerves in cats, the vertical nystagmus upwards is facilitated by tilting head upwards around the bitemporal axis. On the other hand, selective sectioning of the two posterior ampullar nerves induces a vertical nystagmus downwards, which is facilitated by tilting the animal's head downwards. Tilting in the opposite direction inhibits nystagmus in both these cases. Unilateral sectioning of the two vertical ampullar nerves gives a rotatory nystagmus with the upper pole of the eyes beating towards the sound ear. Tilting the animal around its longitudinal axis towards the sound ear facilitates this nystagmus whereas tilting in the opposite direction causes an inhibition. Finally, unilateral selective sectioning of all the three ampullar nerves induces a horizontal rotatory nystagmus. In one lateral position the horizontal component is prevalent, in the other the rotatory.

## CONCLUSIONS

From all these results the following conclusions can be drawn. A unilateral labyrinthine disease can, during tilting around the longitudinal axis of a patient in supine position, induce a horizontal nystagmus, if the patient is tilted towards the affected ear, and a rotatory nystagmus (with the upper pole of the eyes beating towards the sound ear) if the patient is tilted towards the sound ear. These findings coincide completely with clinical results obtained by Barany, Ruttin, Voss and Nylen and are classified as Type 1.

A very important finding of the animal experiments is that a horizontal, positional nystagmus released from the labyrinth invariably beats in the opposite direction to that in which the animal is tilted. These findings also coincide with Nylen's Type 2.

Concerning Type 3 it can immediately be stated that the experimental investigations have



shown a clear relation between position and distinct eye movements. Therefore, if a patient during one and the same investigation manifests an irregular positional nystagmus, this indicates a nonlabyrinthine disease. On this most authors also agree.

Here, it may be appropriate to point out that it is not possible to "reverse" the problem i.e., to draw, from a certain finding, the conclusion that the damage is localized in this or that peripheral labyrinth, since it must always be borne in mind that pathological processes may be present at any place in the reflex arc and still display the same system. This is why Types 1 and 2 are found even in diseases of the central nervous system.

### Definitions

The above-mentioned experimental investigations have now enabled us to indicate an appropriate method for investigating positional and positioning nystagmus, and also to state the results which can be expected from the peripheral labyrinth. However, it is imperative to first clearly define what is meant by these expressions.

1 Positional nystagmus (*Lagenystagmus*) is a static reaction, appearing in certain positions without preceding angular acceleration.

2 Positioning nystagmus (*Lagerungsnystagmus*) is a dynamic reaction, due to combined angular and linear acceleration, or to lymphokinetic movements in the perilymphatic system, stimulating the peripheral sense organs.

### Methods of investigation

The fact that the otolith organs and the semicircular canals are intimately integrated in their activities and their influence on oculomotion, makes certain demands on the method of investigation.

A In order to get answers which are as clean as possible, preferably concerning only one of the rotation axes of the eye, the stimuli must be applied in distinct planes in relation to the sensory cells, which are to be stimulated.

B A clean stimulation of the otolith organs

presupposes the absence of stimulation of semicircular canals. The angular acceleration which brings the patient into the desired position must always be subthreshold. This can only be done by means of electrically-driven rotatable tables, making very slow accelerations possible. Such a table, specially made for the purpose, manufactured by MSA (Stille-Werner).

C Concerning the test for positioning nystagmus the intensity of the stimulus must be known and reproducible.

The most suitable positions for positional nystagmus are

1 Lateral position, which can give rise to lateral or rotatory nystagmus.

2 Supine or prone position, which only gives vertical nystagmus. To place the patient in this head hanging backwards does not involve an increased stimulation of the otolith organs and can, from this point of view, be discarded. Possibly, this position can affect the muscle of the neck or the vertebral arteries or have a lymphokinetic effect via the cochlear aqueduct, thus supply information of a primarily vestibular character.

### Normal and pathological results to be expected from the labyrinth

If the saccule, which causes vertical eye movements (Fluur & Mellstrom, 1970b), is not taken into consideration, our present knowledge of the utricular function entitles us to count on the following results during normal and pathological conditions. The *g* force must be regarded as a vector, which, in different positions, changes direction over the utricular surface (Fig. 1).

## POSITIONAL NYSTAGMUS

### Supine position

*Normal patients* In this position there is an increased activity from the anterior lateral and posterior medial areas of both utriculi, which causes a depression of the eyes. Simultaneously the posterior lateral and the anterior medial areas, which enable elevation, are inhibited. The midlateral areas, which account for horizontal

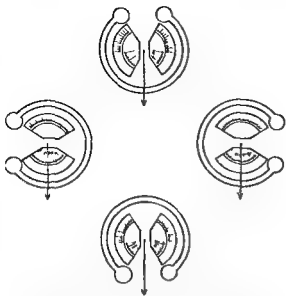


Fig 1 Schematic diagram of the horizontal semicircular canals and the utricles in different positions as seen from above. The small arrows on the utricular surfaces indicate areas of increased activity in a certain position. The large arrows indicate the direction of the  $g$  force.

eye movements, do not have any effect on the eyes, since there is no difference in activity between the two sides.

**Acute unilateral damage of the labyrinth.** Only horizontal nystagmus towards the sound ear is obtained. Vertical nystagmus is not to be expected, because both elevation and depression are represented on only one utricle. On the other hand, unilateral damage can give rise to a rotatory component of nystagmus. However, horizontal nystagmus preponderates over vertical and rotatory nystagmus.

**Compensated unilateral damage of the labyrinth.** No nystagmus or nystagmus only with open eyes in darkness or behind closed eyes and with electronystagmographic recording.

#### Right lateral position

**Normal patients.** Here there is increased activity in the lateral part of the left utricle, and in the medial part of the right (Fig 1). This causes a horizontal deviation of the eyes to the right and, simultaneously, a clockwise rotation.

**Acute unilateral damage of the labyrinth.** If the damage is on the right side, there is an increase

in the frequency of the horizontal, left-beating nystagmus. A left-sided damage causes a counter-clockwise rotatory nystagmus (Nylén, 1950, Fluor & Siegborn, 1973).

**Compensated unilateral damage of the labyrinth.** Here positional nystagmus is horizontal if the damage is right-sided and counter-clockwise rotatory if the damage is left-sided.

#### Prone position

**Normal patients.** Increased input is obtained from the anterior medial and posterior lateral areas of both utriculi (Fig 1), and the patient's eyes are elevated. Simultaneously, the anterior lateral and posterior medial areas, which are responsible for depression, are inhibited. The "horizontal" components are not influenced for the same reasons as in the supine position.

**Acute unilateral damage of the labyrinth.** This causes a spontaneous horizontal nystagmus towards the sound ear, sometimes with a rotatory component with the upper pole of the eyes beating in the same direction.

**Compensated unilateral damage of the labyrinth.** No nystagmus or nystagmus only with open eyes in darkness or behind closed eyes and with electronystagmographic recording.

#### Left lateral position

**Normal patients.** There is an increased input from the lateral part of the right utricle and from the medial part of the left (Fig 1), which causes a horizontal eye deviation to the left and a counter-clockwise rotation.

**Acute unilateral damage of the labyrinth.** This increases the right-beating nystagmus, if the left labyrinth is damaged, or a clockwise, rotatory nystagmus if the damage is on the right side (Fluor & Siegborn, 1973).

**Compensated unilateral damage of the labyrinth.** This induces positional nystagmus, horizontal when the left side is damaged, and clockwise rotatory in right-sided diseases.

## POSITIONING NYSTAGMUS

The term "positioning nystagmus" is commonly used for a nystagmus induced by a rapid alter-

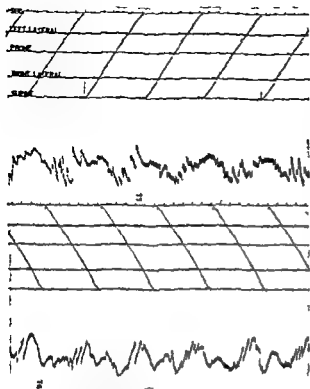


Fig 2 Electro-nystagmographic recordings from a normal person during constant rotation at a speed of 60°/sec around the longitudinal axis. The upper half of the curves indicates the rotation of the table, the lower half indicates the horizontal nystagmus. The upper curves show nystagmus towards the right during clockwise rotation, inhibited in the right lateral position, and facilitated in the left lateral position. The lower curves show nystagmus towards the left during counterclockwise rotation, inhibited in the left lateral position and facilitated in the right lateral position.

of position in the sagittal plane according to Dix & Hallpike (1952), Cawthorne (1954) and Stenger (1955). The positioning procedure used by Stenger with a movement only in the sagittal plane involves a stimulation of the utricles and the two anterior or posterior semicircular canals and ought to give vertical nystagmus. The rotatory nystagmus obtained by him must therefore have another cause. According to the author this movement can give rise to lymphokinetic movements in the perilymph via the cochlear aqueduct, most often producing a rotatory nystagmus of short duration and without reproducibility.

On the other hand, the procedure used by Dix & Hallpike with a movement in the sagittal

plane, combined with a turning of the head to either side, could very well induce such a rotatory nystagmus by an ampullofugal endolymp movement in the two vertical canals of the labyrinth and on ampullopetal movement in same canals of the contralateral labyrinth. Therefore it is an excellent test of the utricular circular integrations in benign paroxysmal positional nystagmus, by the authors supposed have its cause in the utricle.

Sticking to the definition of positioning nystagmus as a dynamic reaction, a slow alteration of position of a supine patient with a rotation around his longitudinal axis introduces a kind of positioning horizontal nystagmus, hitherto not used as a clinical test of the interaction between the semicircular canals and the otolith organs.

We know that the otolith organs *per se*, without the presence of semicircular canals are capable of inducing nystagmus (Fluor & Sborn, 1973d). The otolith organs are dependent on functioning semicircular canals, the input of which is modulated by changing the utricular activity. Therefore, a combined stimulation of the semicircular canals and the otolith organs is a good method for studying the cooperation

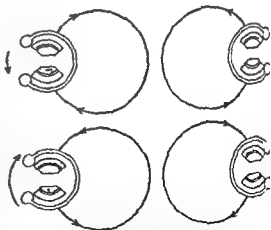


Fig 3 Schematic diagram of the horizontal canals and the utricles, as seen from above, when rotated around the longitudinal axis of the subjects. The circular arrows indicate the direction of rotation. The smaller arrows at the side indicate the intensity and direction of the horizontal nystagmus at this rotation. The small arrows on the utricle indicate areas of increased activity in a certain position.

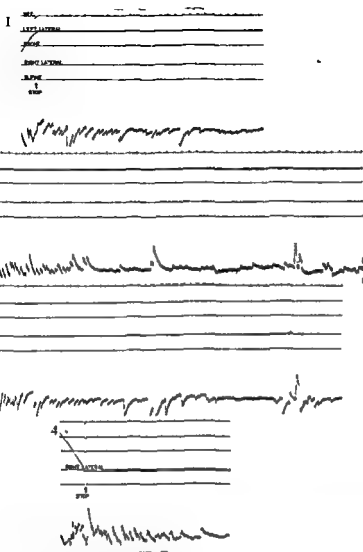


Fig 4 Electro-nystagmographic recording of postrotatory horizontal nystagmus from a normal person when rotation at a speed of 60/sec is suddenly stopped, in left (curves 1 and 2) or right (curves 3 and 4) lateral position after clockwise (curves 1 and 3) or counter-clockwise (curves 2 and 4) rotation. The upper half of

one curve shows the position of the table, the lower half the direction and intensity of nystagmus. In the positions where the utricles and the semicircular canals are functioning synergistically the duration of postrotatory nystagmus is much longer—about twice—than in the positions where they are functioning antagonistically.

between the horizontal semicircular canals and the utricles in their influence on the oculomotion. With an electrically-driven rotation table the patient can, in supine position, be rotated around his longitudinal axis to the right or the left with a reproducible acceleration. Through the simultaneous alteration of the direction of the  $g$  vector on the utricular surface the input from the utricle modulates the nystagmus induced from the hori-

zontal canals. In the supine position there is indeed an influence on those sensory cells which cause vertical eye movements, but during increased tilting, the effect of the  $g$ -force increases with the sine of the angle on those cells which give rise to horizontal or rotatory eye movements. The output from the sensory cells increases namely with the cosine of the angle between the direction of applied displacement of

the sensory cells and the direction of maximum sensitivity (Flock, 1965). In supine position, these cells are in a "spontaneous" position, whereas in a lateral position they are in "optimal" position.

If the patient is accelerated to the right, nystagmus beating to the right occurs. In the right, lateral position an antagonism develops between the input from the left utricle and the right horizontal canal which somewhat inhibits the nystagmus. In the left lateral position, on the other hand, the input from the right utricle and right horizontal canal functions synergistically, which increases the intensity of the right-beating nystagmus (Fig. 2). The table can also be stopped after moving at a constant speed to the right or left (Fig. 3). Then, by stopping in a right lateral position after rotation to the right postrotatory nystagmus to the left is developed in the patient, which is facilitated by the simultaneously increased input from the left utricle. If the table is stopped in the left lateral position after rotation to the right, the postrotatory nystagmus to the left is inhibited by the increased input from the right utricle. By comparing the results from the right and the left lateral position after rotation in both directions, information is obtained about the integration of the activity between the utricles and the horizontal canals (Fig. 4).

If there is any value in investigating the interaction between the utricles and the vertical canals, this can be done by rotating the patient in the sagittal plane from a sitting position at a known acceleration forwards or backwards, to a horizontal position. In this case the utricles and the vertical canals are operating synergistically, producing a clean simultaneous angular and linear stimulation, which under normal conditions results in vertical nystagmus. Since the activity of the cells which cause horizontal eye movements is on the same level bilaterally, horizontal nystagmus is never obtained by this stimulation.

The recording of the eye movements is a question of utmost importance, since it is impossible to record rotatory eye movements electronystagmographically. Visual inspection of the eyes

behind Frenzel's glasses in darkness is therefore recommended, together with nystagmography recording of horizontal and vertical nystagmus. However, a disadvantage in recording vertical nystagmus is that closing the eyes can not record vertical nystagmus by itself without vestibular stimulation, which makes it more difficult to interpret the nystagmus from the labyrinth (Fluor & Mendel, 1963). Therefore such recording ought to be done with open eyes in the dark.

## ZUSAMMENFASSUNG

Es wird eine kurze Übersicht über das Problem Lagenystagmus gegeben. Die verschiedenen Positionen zur Hervorbringung eines Lagenystagmus werden beschrieben und die verschiedenen Typen diskutiert. Ergebnisse von Versuchen an Tieren und Menschen während der letzten Jahre werden vorgelegt und die klinischen Schlüsse gezogen. Lage- und Lagerungsnystagmus wird definiert und die geeignetsten Positionen für Lagenystagmus genannt. Normale und pathologische Ergebnisse bei diesen Positionen vom Labyrinth zu erwarten sind, werden dargelegt. Eine Methode, die den horizontalen Lagerungsnystagmus als Ergebnis einer integrierten utriculocularen Aktivität studiert, wird beschrieben und die Ergebnisse werden diskutiert.

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## HISTOCHEMICAL LOCALIZATION OF ACETYLCHOLINESTERASE ACTIVITY IN THE COCHLEAR AND VESTIBULAR GANGLION CELLS

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(Received December 9 1973)

**Abstract** The localization of AChE activity was investigated in the cochlear and vestibular ganglion cells of the chinchilla inner ear with the electron microscope by Karnovsky's method. The reaction product was localized within the rough surfaced cisterns of the E.R. including the perinuclear cistern and in some cisterns of the Golgi complex. In a few myelinated nerve fibres and in most of the unmyelinated ones the reaction product was present along the axolemma. The reaction product was absent in the satellite and Schwann cells. The controls indicated that the reaction was due to AChE activity.

After Churchill & Schuknecht (1959) and Schuknecht et al (1959), numerous other investigators confirmed, both at light and electron microscopical levels, that in the organ of Corti and in vestibular sensory areas, acetylcholinesterase (AChE) activity is strictly associated with the afferent nerve fibres and endings. Under the same experimental conditions, the afferent nerve endings did not show any AChE activity (reviewed by Iurato et al., 1971a).

In the cochlear and vestibular ganglion cells, which give origin to the afferent nerve endings, resulted negative at the periphery, some authors found evidence of AChE activity, but contradictory results were obtained by others. In the cochlear ganglion cells Vinnikov & Titova (1964), Firbas & Welleschik (1970), Nakai (1972) and

Firbas et al (1973) found a positive reaction. Schuknecht et al (1959) obtained a negative result, but they could not conclude that structures were free of AChE activity for solutions probably did not penetrate to the ganglion. Rossi (1961) found a positive reaction in the cells of the cochlear ganglion in the guinea pig, only in the early foetal development. Schuknecht et al (1967a) demonstrated a positive AChE action in the saccular and cochlear ganglion of the human inner ear, but not of the mouse, cat, guinea pig (1967a) and squirrel monkey (1967b).

On the other hand, in other sensory ganglia (spinal and trigeminal) of adult and young animals, AChE activity was demonstrated by using the light microscopic histochemical methods, electron microscopic histochemical methods and microchemical techniques (reviewed by Pannese et al., 1971, 1974).

The present research was undertaken to investigate whether the neuronal bodies of cochlear and vestibular ganglion cells show in the chinchilla AChE activity with a method (Karnovsky's) which already gave clear-cut results in the organ of Corti and in vestibular sensory cells of the same zoological species (Iurato et al., 1970, 1971a, 1971b).

In case of positive result it was of some interest to establish the precise sites of AChE activity in these neuronal bodies.

Research financed partly by the Consiglio Nazionale delle Ricerche grants Nos CT 72.00775.04.115.227 and 71.00863.04.115.1144 and partly by the Deutsche Forschungsgemeinschaft grant No. RE 257/E.

Additional objectives of the research were to check the behaviour in respect of AChE activity of the two cell populations described in the cochlear (Kellerhals 1967) and vestibular (Ballantyne & Engstrom 1969) ganglia and to control the existence of connections between the nerve cells and of synapses in the same ganglia. In fact Adamo & Daigneault (1972) found finger-like intercellular projections connecting the neurons in the cochlear ganglion of the cat and Brenbrand & Wittemann (1970) described synaptic nerve endings in contact with the vestibular ganglion neurons in the mouse structures not mentioned in the previous extensive studies by Kellerhals (1967) and Ballantyne & Engstrom (1969).

## MATERIAL AND METHODS

Five adult chinchillas were anesthetized with an intraperitoneal injection of sodium pentobarbital (15 mg/kg). Artificial respiration was maintained using a Braun Melsungen apparatus. The animals were first endovascular perfused with 5 ml heparin (pH = 7.2) then with a fixative containing formaldehyde 2% and glutaraldehyde 2.5% in buffered 0.1 M sodium cacodylate (pH 7.3) with 10 mg%  $\text{CaCl}_2$ . Immediately afterwards the temporal bones were quickly removed. The bony cochlea was widely opened and the stapes taken away to allow a good penetration of the fixative. The specimens were left 2 hours in the same fixative used for the perfusion. After the fixation was completed the specimens were placed in the same previously used buffer containing 0.22 M sucrose for 2 hours and the dissection of the cochlear and vestibular ganglia was performed.

### Morphology

Some specimens containing cochlear ganglion cells and others with the vestibular ganglion were post fixed for 2 hours with 1% osmium tetroxide buffered according to Millonig (1961).

### Histochemistry

From other specimens 75  $\mu\text{m}$  thick sections were prepared using a Sorvall TC2 tissue chopper.

These sections were rinsed in 0.44 M sucrose for 20 minutes then transferred into the incubation medium (substrate acetylthiocholine (AThCh) iodide Hoffmann La Roche & Co Ltd Basel or Fluka AG Bucks St Gallen) prepared according to Karnovsky (1964). The incubation time was 30 minutes. As a control for the histochemical reaction a number of thick sections was preincubated for 1 hour in a  $2 \times 10^{-4}$  M aqueous solution of BW 284 C 51 ([1,5-bis(4-allyl dimethylammoniumphenyl)pentan-3-one dibromide] Wellcome Research Laboratories Beckenham Kent England). These sections were then incubated for 30 minutes in a complete medium to which the inhibitor was added at the same concentration used for the pre incubation.

After incubation all thick sections were rinsed in 3 changes of 0.44 M sucrose for 10 minutes. They were post fixed for 30 minutes in 1% osmium tetroxide buffered according to Millonig (1961) and then dehydrated in alcohol. All these procedures were performed at 0–4°C. Epon 812 was used as embedding medium. Thin sections were cut with an LKB 4800 III ultramicrotome. The thin sections were placed on Formvar carbon membranes (Dowell 1964) or on Formvar grids and observed with Siemens Elmiskop 10I and 1A and Zeiss EM 9A electron microscopes.

## RESULTS

### Localization of reaction product

Since the localization was similar in the cochlear and vestibular ganglion cells we will give a unique description as given. In the neuronal body most of the reaction product (Cu ferrocyanide) was localized within the rough surfaced cisterns of the endoplasmic reticulum (ER) including the perinuclear cistern (Figs 1, 2a, 2b). Reaction product associated with dense bodies was not observed. Golgi complexes showing reaction product could be observed (Fig 2c) together with Golgi complexes devoid of reaction product in the same section of a neuronal body. The reaction product was never observed along the perikaryal plasma membrane and the plasma membrane of the initial portions of the dendrite.





*Fig 1* Vestibular ganglion cell from a chinchilla. The nucleus (N) is located centrally and contains one nucleolus (Nu). The surrounding cytoplasm contains rough surfaced cisterns of the endoplasmic reticulum and large numbers of organelles. The cell is surrounded by a multi-

layered myelin sheath, consisting of both compact and loose myelin (MyL). S - satellite cell. C - cleft space. UF - unmyelinated nerve fibres. The product is localized within the rough surfaced of the endoplasmic reticulum.  $\times 10,800$

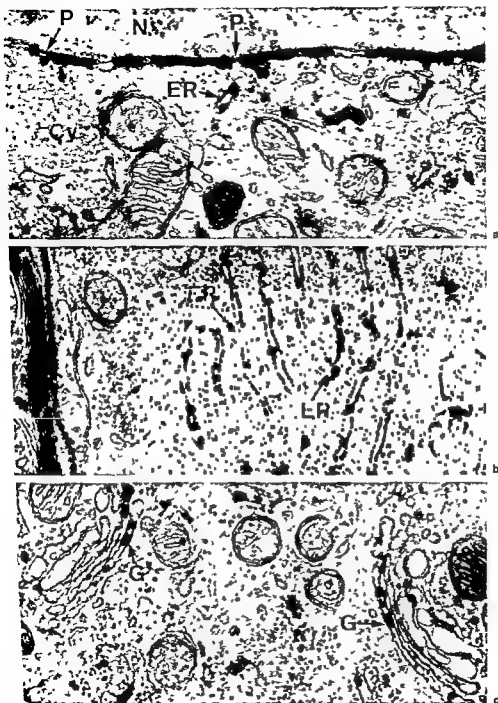


Fig. 2 (a) Detail of a chinchilla vestibular ganglion cell showing the nucleus (N) and a small part of cytoplasm (P). P pore in the nuclear envelope. The reaction product is localized within the perinuclear cisterna and in a tern of the rough endoplasmic reticulum (ER)  $\times 52000$  (b) Detail of a chinchilla cochlear ganglion cell, surrounded by the myelin sheath (M). The granules of the

reaction product appear localized within the rough-surfaced cisterns of the endoplasmic reticulum, arranged in parallel arrays (ER)  $\times 44000$  (c) Detail of a chinchilla vestibular ganglion cell showing two Golgi complexes (G). The reaction product appears within some of the Golgi cisterns  $\times 50000$



Fig 3 (a) Detail of the cochlear ganglion from a chinchilla showing two unmyelinated axons (A), containing micro tubules and filaments Af; myelin, Sch - Schwann cell, GJ - gap junction, Bm - basement membrane. The

reaction product is localized along the axolemma  $\times 18\ 000$  (b) Myelinated fibre in the cochlear ganglion from a chinchilla. The reaction product is localized the axolemma  $\times 62\ 000$

axon<sup>1</sup> In a few myelinated nerve fibres a small amount of reaction product was present along the axolemma (Fig 3b). Most of the unmyelinated nerve fibres showed a large amount of the reaction product on their outer surface (Fig 3a). The satellite and Schwann cells did not contain any reaction product. In agreement with Pannese et al (1974), who used the same technique to study the spinal ganglia of adult fowls, the above described pattern of localization of the reaction product could only be observed in the more superficial layers of the thick sections obtained with the tissue chopper, whereas in the innermost layers of the thick sections neuronal bodies devoid of reaction product could be found. In the innermost layers of the thick sections the reaction product was present in the cleft between the neuron plasma membrane and the related satellite cell sheath as well as in the cleft between the satellite cells.

#### Controls

In any of the above mentioned sites no reaction was obtained in the presence of  $2 \times 10^{-4}$  M BW 284 C 51. As BW 284 C 51 is an AChE selective inhibitor (Austin & Berry, 1953; Bayliss & Todrick, 1956) further controls were not carried out.

### DISCUSSION

In both the cochlear and vestibular ganglion the reaction was positive in the following sites: rough surfaced cisterns of the E.R., including the endoplasmic cistern and part of the Golgi complex of the ganglion cells; axolemma of a few myelinated and of most of the unmyelinated nerve fibres. Since the reaction was positive by using AThCh as a substrate without inhibitors and negative by adding BW 284 C 51 as an inhibitor it can be concluded that it was due to AChE activity.

In the present research the reaction was per-

According to most of the Authors in this paper the term dendrite is used to refer to the process of the cochlear and vestibular ganglion neurons directed peripherally and the term axon is used to refer to the process directed centrally.

formed on 75–100  $\mu$ m thick sections to allow the solutions to penetrate the ganglion and the myelin sheath of the ganglion cells. In our material neuronal bodies which did not present any reaction product were observed only in the innermost layer of the sections. On the basis of the results obtained, it is possible to conclude that AChE activity is present in the neuronal bodies of the cochlear and vestibular ganglion cells of adult chinchillas like in the sensory ganglia neurons of most adult animals (reviewed by Pannese et al, 1971, 1974). Therefore the negative results obtained in other species by Schumknecht et al (1959), Rossi (1961) and Ishii et al (1967a, 1967b) could be possibly explained with the difficulties the solutions have to penetrate the ganglion and the myelin sheath of the ganglion cells.

The sites where AChE activity was demonstrated in the present research are the same found by numerous authors in the sensory ganglia and in other regions of the nervous system (reviewed by Pannese et al, 1974).

Both the myelinated and the unmyelinated ganglion cells in the vestibular ganglion of the chinchilla showed the reaction product. Therefore no difference in respect of AChE activity was evidenced in the two cell populations of the vestibular ganglion. In the cochlear ganglion of the chinchilla we did not find a single unmyelinated cell, but this is probably due to the limited number of sections observed.

The morphological controls fail to demonstrate any type of contact between the cochlear ganglion cells and of synapses around the vestibular ganglion cells. Our results are therefore in agreement with those of Kellerhals (1967) and Ballantyne & Engstrom (1969) and not with those of Adamo & Daigneault (1972) and Ehrenbrand & Wittemann (1970). Synapses were found in the vestibular specimens only when the section interested the connection between the vestibular nerve and the brain stem and therefore contained some neurons belonging to the latter.

The problem of the histochemical properties of the ganglionic nerve fibres was not solved. As shown by Iurato et al (1971a) and Pannese

et al (1974) reliable results can be obtained at an electron microscopic level only with serial sections. Since this was not done in this study, no conclusions could be drawn. Moreover the thick sections cut by the tissue chopper did not permit the identification of the intraganglionic spiral bundle which was found definitely positive by Nomura & Schuknecht (1965).

Since AChE activity was demonstrated in the neuronal bodies of cochlear and vestibular ganglion cells but not in the peripheral (Iurato et al, 1970, 1971a, 1971b) nor in the central (McDonald & Rasmussen, 1971) terminals of these neurons, the role of this enzymatic activity remains unclear. It must be taken into account that in several zoological species AChE activity has been definitely demonstrated in spinal ganglion neurons which are regarded as non cholinergic in nature (Eccles, 1964, Bremer, 1953, Feldberg, 1954). On the role of AChE activity in the neuronal bodies of cochlear and vestibular cells the following hypotheses can be put forward:

(1) The cochlear and vestibular ganglion cells are non cholinergic neurons and AChE activity has only a "vestigial" significance (Koelle, 1955, Feldberg, 1957).

(2) AChE activity in these neurons plays the universal role of restoring membrane permeability to the resting state in the conducting cells (Burn & Rand, 1959, 1963).

(3) AChE plays an intermediate part in the synthesis of the actual transmitter, whether it is ACh or not (Burn & Rand, 1959, 1965, Koelle, 1963, Eränkő, 1967).

On the basis of the information available up to now it is not possible to determine which of the above-mentioned hypotheses is more likely to be the correct one.

## ACKNOWLEDGEMENT

We wish to express our thanks to Miss Carole Davidson for her help in checking the English style of the manuscript.

## ZUSAMMENFASSUNG

Der histochemische Nachweis der Acetylcholinesterase Aktivität in cochlearen und vestibulären Ganglienzellen

des Innenohres von Chinchillas wurde unter Verwendung der Karnovskyschen Methode durchgeführt. Das

produkt am Axolemm sichtbar. Es fehlte in Satelliten und Schwannschen Zellen. Kontrollversuche bestätigten die Spezifität der Reaktion auf Acetylcholin Aktivität.

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## THE VESTIBULAR AQUEDUCT IN PATIENTS WITH MENIÈRE'S DISEASE

### *A Tomographic and Clinical Investigation*

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(Received October 25 1973)

**Abstract** The vestibular aqueduct was identified on tomograms in all healthy subjects. In patients with severe Menière's disease the visibility was affected. The aqueduct was visible in 65% of the non-diseased ears and in 59% of the diseased ears of patients with unilateral Menière's disease, whereas the corresponding figure for bilaterally diseased ears was only 53%. Longstanding disease can impair the visibility, while it does not seem to be affected by advanced age *per se*. No obvious relationship was found between the visibility on tomograms and the results of hearing tests and caloric tests. The vestibular aqueduct is shorter in patients with Menière's disease than in healthy subjects. The pneumatization of the pyramid, which varies in normal individuals as well as in patients with Menière's disease, influences the length of the aqueduct. The longest aqueducts in healthy subjects were found in those with large cell pneumatization in vicinity of the aqueduct. Most patients with Menière's disease lacked pneumatization of the pyramid and the aqueducts were found amongst them. The aqueduct and the adjacent para vestibular sacculus have been demonstrated by means of plastic moulding. Minute communicating channels probably housing parts of the blood supply to the endolymphatic duct and sac, have been revealed. The para vestibular canaliculus which lies at the borderline of the tomographically demonstrable structures could be discerned in the tomograms of 10 of the 32 ears of healthy individuals and 10 of the 86 ears of patients with Menière's disease.

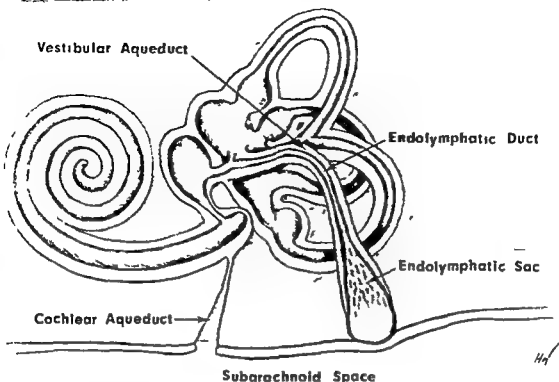
A well functioning endolymphatic duct and sac is said to be a pre-requisite for normal labyrinthine function. Experimental studies have indicated that the main function of the endolymphatic sac is to act as a resorptive and defen-

sive mechanism for the inner ear (Lund 1965). Functional ablation of the sac leads to endolymphatic hydrops (Kimura & Schuknecht 1965, Kimura, 1967, Schuknecht et al 1967). The rapidity of the onset and severity of hydrops following ablation of the endolymphatic sac varies with the species (Yuen & Schuknecht 1972).

Structural abnormalities of this duct and impairing their resorptive function, are thought to be a major factor in the pathogenesis of Menière's disease (Shambaugh et al, 1969). Resorption of endolymph might be disturbed through a deficiency of its circulation in the duct, due to narrowing of the vestibular aqueduct. This latter can be caused by the formation of subepithelial concretions, which in time can be transformed to bone, resulting in "chronic retention hydrops" in the inner ear (Witma 1956). In Menière's disease exostosis like growths of lamellar bone on the walls of the aqueduct have been described by Zechner & Altmann (1969) and Zechner (1973). Complete blockage or plugging in sporadic cases has been reported (Clemis & Valvassori, 1968).

The function of the duct and sac may be interfered with by subepithelial avascular fibrous tissue producing ischaemia of the sac wall (Shambaugh et al, 1969). Such perisaccular fibrous tissue was first described by Hallpike & Cairns (1952) in patients with Menière's disease as well as in normal subjects (Zechner & Altmann (1969).

This study was supported by the Medical Faculty of the University of Uppsala and the Swedish Medical Research Council (Project No. B74-17X-3908-02).



1 Schematic drawing of the endolymphatic and perilymphatic systems and their relation to the subarachnoid space

However, only observed such fibrosis in specimens from persons with Meniere's disease. Results contradictory to the above-mentioned findings have been reported in two recent papers. Yuen & Schuknecht (1972), after histological examination of temporal bones from 19 patients with Meniere's disease, concluded that the vestibular aqueducts in Meniere's patients were not different in calibre from those of normal ears. The endolymphatic ducts, on the other hand, were found to be narrower than those in the control group. Gussen (1973), describing one patient, stated that the endolymphatic duct and intermediate portion of the sac of the diseased side had a rather straight course to the superior fossa with virtually no lateral curving. Otherwise it appeared normal, with a small amount of loose tissue about its intermediate portion. The duct and sac on the opposite, healthy side had the same appearance.

The aim of our investigation was to study the tomographic visibility and length of the vestibular aqueduct in (a) a group of healthy subjects and (b) a group of patients with very disabling Meniere's disease. The visibility was correlated to clinical data in an attempt to assess the diagnostic value of the radiographic examination. The pneumatization of the aqueductal region was correlated to anatomic and clinical findings. In addition an attempt was made to find the para vestibular canaliculus, this has not previously been identified radiographically either in normal subjects or in patients.

#### *General morphology and previous tomographic investigations*

The vestibular aqueduct originates from the medial wall of the vestibule passing through the otic capsule immediately in front of and somewhat medial to the *crus commune* (Figs 1 and





Fig. 2 Temporal bone preparation demonstrating the courses of the vestibular and cochlear aqueducts (black-stained). The proximal portion of the vestibular aqueduct parallels the crus commune (CC) of the superior and inferior semicircular canals. The peripheral portion is a flat, triangular widening. A small bony ridge is

left to mark the external aperture of the vestibular duct (right arrow). The distinct round opening (s) corresponds to the canal for the posterior auricular nerve. The left arrow points at the external opening of the cochlear aqueduct. MAI, internal acoustic meatus; SS, sigmoid sinus.

It then curves (Figs 2 and 3), forming a more or less pronounced angle on its course to the external aperture on the cerebellar surface of the petrous bone (Bast & Anson, 1949; Wilbrand et al., 1974). The external opening, which in most instances can be recognized about 10 mm posterolateral to the *porus acusticus internus* and 10 mm inferior to the *sulcus petrosus superior* (Anson, 1965; Anson et al., 1968) may show great individual variations. It can be more or less sheltered by a bony ridge (Figs 2 and 7). The endolymphatic sac is partly housed in the vestibular aqueduct. The *pars rugosa* of the sac occupies the flat, triangular distal part, while the extraosseous portion lies intradurally close to the sigmoid sinus (Bast & Anson, 1949). The ratio of the intra-osseous to the intradural part

of the sac is man varies with the degree of pneumatization of the pyramid (Wittmaack, 1964). Rich pneumatization is said to be accompanied by a long intraosseous and a short intradural part. Sparse or absent pneumatization, on the other hand, should mean a short intraosseous and a long intradural part. These anatomical variations may contribute to the difficulties reported in identifying the sac at drainage operations (House, 1964).

The arterial blood supply of the vestibular aqueduct and its content is still unclear (Figs 2 and 3). Bast & Anson (1949) have described several branches from the posterior meningeal artery supplying the dura around the endolymphatic sac, and accompanying the duct for a short distance. Small veins form a plexus around

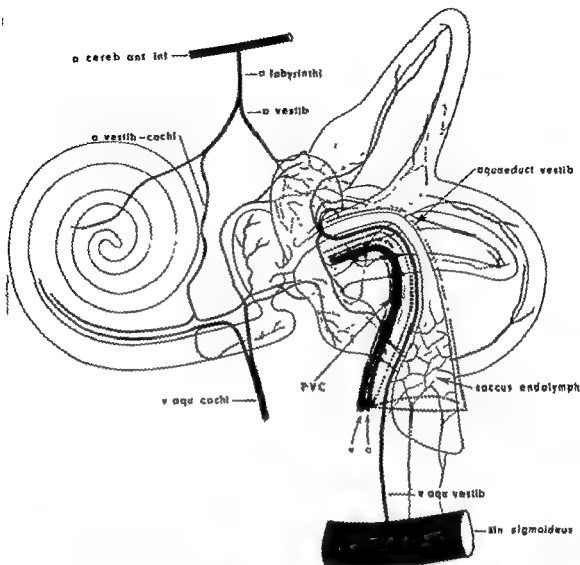


Fig. 3. General survey of the vascular supply of the vestibular part of the human inner ear. The cochlear vascularization has been purposely neglected. The arterial supply takes place through the vestibular artery and by its branches of the vestibulocochlear artery. The

venous drainage occurs partly by branches to the vein in the cochlear aqueduct and partly by the veins in the vestibular aqueduct ending in the sigmoid sinus. The shaded area, P.V.C., represents the para vestibular canalculus housing an artery and vein.

istal widening part of the aqueduct. The venous blood from the non-acoustic labyrinth is mainly collected to the vein of the vestibular aqueduct (Jeck, 1965). This vein receives numerous small veins from the plexus around the endolymphatic sac before it finally drains into the sigmoid sinus (Bast & Anson, 1949).

Tomographic reproductions and descriptions of the aqueduct have been published by Clemis

& Valvassori (1968), Wilbrand (1971), Br  nner & Pedersen (1971), Sesana et al (1971) and Bisch & Barrionuevo (1972). Wilbrand et al (1974) found that in tomograms of temporal bone specimens the entire aqueduct was visible in 51% of the cases. On subsequent microdissection the aqueduct was identified in all specimens. The inability to visualize all aqueducts tomographically was considered to be mainly due to

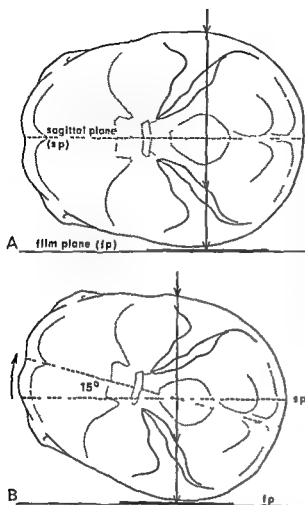


Fig. 4. Schematic drawing of tomographic positioning applied in this investigation. (A) True lateral projection, Slight elevation of the face (15°) from the true lateral position.

adequate positioning of the specimens. In a clinical study Clemis & Valvassori (1968) reported that in patients with hearing loss of the conductive type they were able to identify the aqueduct on tomograms in more than 90%, while in 28 out of 35 patients with Meniere's disease it could not be identified. On the basis of these findings Arenberg et al. (1970) have recommended decompression surgery of the endolymphatic sac only in those patients in whom the vestibular aqueduct has been found to be open on tomography.

#### *The para-vestibular canaliculus*

In 1971, Ogura & Clemis discovered on microdissection a tiny bony canal which they design-

nated the para-vestibular canaliculus. It was, after osmic acid fixation in 15 out of 23 specimens, running from the vestibule posterior cranial fossa, in some cases proximity to the vestibular aqueduct, others a certain distance away. The canal contained an artery and a vein, which they thought to constitute the blood supply of the endolymphatic sac. They also attributed to the canaliculus the function of a pressure equalizing valve between the perilymphatic space and the posterior fossa. Wilbrand et al. (1977) studied 35 human temporal bones and were able to identify the canaliculus on tomograms in seven of the specimens. On submicrodissection the osmic acid stained canaliculus was verified in all specimens. Distal canaliculi were observed as well as variations in the course. The morphology and function of the blood vessels lodged within the para-vestibular canaliculus have not yet been evaluated.

## MATERIAL AND METHODS

The investigation was made on

(a) Twenty healthy persons between 15 years of age (mean age 33 years). Both ears were tomographed in 12 subjects and one ear in the remaining eight, making a total of 24 examined ears.

(b) Forty-three patients (12 women and 31 men) with severe Meniere's disease. They were between 22 and 76 years old (mean age 48 years). According to the hearing tests the disease was unilateral in 33 and bilateral in 10 patients. All ears (86) were tomographed. The age at onset of the disease was between 10 and 60 years (mean age 41 years). The duration of the disease at the time of the investigation was between 1 and 30 years (mean 7.7 years). The pure tone threshold of the diseased ears was between 18 and 100 dB (mean 53 dB), the discrimination scores for the same ears being 0-100% (mean 61%). The caloric response assessed by electronystagmograms was reduced in 55% of the patients with unilateral disease, the average difference in excitability (maximum speed

Table I The visibility of the vestibular aqueduct in tomograms, expressed as the percentage number of ears with visible aqueducts

	Patients with Meniere's disease (86 ears)		
	Unilaterally diseased		
Healthy subjects (2 ears)	Non diseased ear	Diseased ear	Bilaterally diseased
100%	65%	59%	53%

low nystagmus phase) was 32%. Among the 11 patients with bilateral disease the caloric response was abnormal in eight and normal in three.

Tomography was carried out with the Philips-Iassiot Polytome, using its hypocycloidal movement at a magnification of 1.3. A focal size of 3 mm was used. The exposure data were 55 kV, 50 mA and 11.6 sec (double hypocycloidal movement). The radiographic system consisted of a cassette with high definition screens and a RPL film. The technique thus corresponded to that used in previous investigations (Sandstrom & Wilbrand 1971, Dahlin et al. 1973, Wilbrand et al. 1974, Wilbrand, 1974).

On the basis of earlier experience (Wilbrand et al. 1974) the subjects were tomographed (1) in the true lateral position and (2) in a position with the face elevated 10–20° from the true lateral position (Fig. 4). In no case was correction of the positioning needed in order to visualize the proximal portion of the vestibular aqueduct. A distance between the cuts of 0.5 mm was used so as to guarantee overlapping of the information content of every cut.

✓ Evaluation of the individual tomograms included (1) repeated checks of the visibility of the true aqueduct, (2) measurement of the proximal and peripheral portions of the aqueduct and (3) grading of the pneumatization in the vicinity of the aqueduct. The tomograms were viewed under optimal conditions (Rohler 1967). Apart from magnifying glasses no further image quality enhancing procedure was employed. The

measurements were performed by means of a vernier caliper (division 1/10 mm).

The material was processed by an IBM 370 Model 155 computer at the Data Center of the University of Uppsala (UDAC).

## RESULTS

1 The vestibular aqueduct was visible on the tomograms of all healthy subjects (32 ears) (Table I, Fig. 5). The mean total length from the internal or vestibular aperture to the external aperture was 8.7 mm (6.1–13.5 mm) (Table II).

2 In patients with unilateral Meniere's disease (66 ears), the aqueducts were visible on the non-diseased side in 65%, and on the diseased side in 59%, of the ears. In bilateral cases this figure was reduced to 53% of the ears (Table I).

3 The total length of the aqueduct in unilateral cases of Meniere's disease (Table II) was 5.1–11.7 mm (mean 7.3 mm) on the non-diseased side and 4.6–10.2 mm (mean 6.8 mm) on the diseased side. In patients with bilateral disease the total length varied between 4.3 and 9.8 mm (mean 6.7 mm).

4 The tomographic visibility of the aqueducts was correlated to several clinical characteristics (Table III). When comparing the mean values, no correlations were found between visibility/non visibility, on the one hand, and age, pure tone threshold, discrimination score or caloric response, on the other. The impairment of the inner ear function in the patients with Meniere's disease, taken as a whole, thus seemed to be of

Table II The length of the vestibular aqueduct, measured on tomograms

	Patients with Meniere's disease		
	Unilaterally diseased		
Healthy subjects (32 ears)	Non diseased ear (26 ears)	Diseased ear (21 ears)	Both ears affected (14 ears)
8.7 mm (6.1–13.5)	7.3 mm (5.1–11.7)	6.8 mm (4.6–10.2)	6.7 mm (4.3–9.8)



Fig. 5 Tomograms of three healthy subjects demonstrating variations in the pneumatization of the pyramid. The longest aqueduct is seen in the most pneumatized pyramid (uppermost picture). Notations in the schematic

drawings: 2 tympanic space, 9 vestibule, 9d v aqueduct, 11 superior semicircular canal, 12 inferior semicircular canal, 20 tympanic ring, P pneuma

### III Tomographic visibility of the vestibular aqueduct in patients with Meniere's disease, correlated to various clinical factors

arterial	Variable mean values	Vestibular aqueduct		)
		Visible	Non visible	
II ears	Age years	47.8 (n 47)	49.7 (n 37) <sup>a</sup>	0.86
	Age at onset years	41.2 (n 47)	41.1 (n 32)	0.97
	Pure tone threshold dB	28.3 (n 47)	35.0 (n 37)	0.97
	Discrimination score %	79.1 (n 47)	73.9 (n 32)	0.71
	Caloric response diff in %	26.1 (n 47)	34.1 (n 37)	1.3
diseased ears only	Duration of disease years	6.7 (n 27)	10.7 (n 26)	1.96 <sup>b</sup>

Seven ears excluded for technical reasons

<sup>a</sup> significant  $P = 95\%$ .

same extent irrespective of the radiographic visibility of the vestibular aqueduct. A significant correlation ( $P = 95\%$ ) was noted between the visibility and the duration of the disease, when only diseased ears were taken into account, the duration being longer in patients with a non visible aqueduct.

5 The pneumatization of the pyramid observed on tomograms varied both in healthy subjects and in patients with Meniere's disease. On the basis of observations on the healthy subjects three main groups could be discerned, designated 1, 2 and 3 (Figs 5 and 6). Group 1 consisted of ears with large cell pneumatization in the vicinity of the aqueduct and group 3 ears without pneumatization, whereas group 2 included ears with varying types of pneumatization, mostly of small cells. The data are presented in Table IV, which reveals an abnormal pneumatization pattern in Meniere's disease, characterized by a high frequency of non pneumatized pyramids.

The total lengths of the aqueducts obtained in Table IV. The pneumatization of the petrous portion of the temporal bone as assessed by tomography (figures indicate the number of ears)

	Group 1 Large cell pneumatization	Group 2 Small cell pneumatization	Group 3 No pneumatization
Normal subjects (33 ears)	11 (34%)	14 (44%)	7 (22%)
Meniere's disease (86 ears)	0	28 (26%)	58 (74%)

from the tomograms in relation to the degree of pneumatization are presented in Table V. In group 1 with large cell pneumatization of the pyramid the aqueducts were longer than in group 3 with no pneumatization. The difference in length was mainly due to a long peripheral portion in the former group. The mean length of 10.3 mm in healthy subjects of group 1 (Table V) exceeded the mean value for the ears of all healthy subjects which was 8.7 mm (Table II). The shortest aqueducts were found in those patients with Meniere's disease with no peripheral aqueductal pneumatization.

6 No significant correlation was found between the different grades of pneumatization and the visibility of the aqueduct in patients with Meniere's disease.

7 The external aperture of the aqueducts varied in shape and width depending upon the pneumatization. Bony ridges sheltering the opening were found in large cell pneumatized pyramids. In non pneumatized bones on the other hand the aqueducts sometimes opened out freely (Figs 5 and 7).

8 The para vestibular canaliculus was visible on the tomograms of 10 out of 32 healthy ears. With reference to the pneumatization of the pyramids four of these ears belonged to group 1 and three to each of groups 2 and 3. The canaliculus was observed in 10 of the 86 ears of patients with Meniere's disease. This structure was also demonstrated by means of plastic filling in temporal bone specimens (Fig 8).

Table V *Pneumatization and length of the vestibular aqueduct in healthy subjects and in patients with Meniere's disease*

Grading of pneumatization of the pyramid	Healthy subjects Total length of aqueduct (32 ears)	Patients with Meniere's disease Total length of aqueduct (86 ears)
Group 1—large cell pneumatization	10.3 mm (7.2–13.5) 11 ears	No patients
Group 2—small cell pneumatization	8.2 mm (6.1–9.9) 14 ears	7.7 mm (5.9–11.7) 28 ears
Group 3—no pneumatization	7.4 mm (6.7–8.1) 7 ears	6.9 mm (4.3–10.2) 58 ears

## DISCUSSION

There is no doubt about the existence of definite differences in the radiographic visibility of the vestibular aqueduct when comparing healthy subjects and patients with Meniere's disease. While the aqueduct was visible in its entire length in all healthy subjects, it was observed in only 59% of the diseased ears in patients. In bilateral cases this figure was reduced to 53%.

The mean whole length of the aqueduct in normal persons, measured on the tomograms in this investigation, was 8.7 mm (range 6.1–13.5 mm). This figure may be compared with earlier values obtained by our group (Wilbrand et al., 1974) in other types of studies, namely 8.1 mm (range 5.7–10.9 mm) in measurements on temporal bone specimens and 8.5 mm (range 6.1–6 mm) in direct measurements on dissected mens. The latter values agree well with is presented by Anson et al. (1965) and Gura & Clemis (1972).

The length of the entire aqueduct, measured on tomograms, was found to be shorter in ears

affected by Meniere's disease than in normal ears. On the whole, the shortest aqueducts were noted in patients with bilateral Meniere's disease. The mean length for that group was 6.7 mm (range 4.3–9.8 mm), which may be compared with the length in healthy subjects, viz. 8.7 mm (range 6.1–13.5 mm). This difference is statistically significant at the 99% level.

Up to now only one major study has been published in which the radiographic findings have been compared with the inner ear function. Thus Clemis & Valvassori (1968) have found a high correlation between abnormal hearing and a non visible aqueduct. We have compared the pure tone threshold, the discrimination index and the caloric response with the radiographic results without finding any obvious relationship (Table III). In other words, an apparent hearing loss, for example, is not necessarily accompanied by a non visible aqueduct. We agree with the opinion of Arenberg and others that any type of drainage operations on the sac in patients with a radiographically non visible aqueduct is probably meaningless because they would not produce an inner ear hydrops. House & Owens (1971) in a long term follow up on patients with Meniere's disease treated with subarachnoid shunt reported failures in 38%, which they attributed among other things, to closure of the lymphatic duct. Preoperative tomography of the vestibular aqueduct therefore seems to be of importance when planning operations on the sac.

The relationship between the radiographic results and the age of the patient was also studied.



Fig. 6. Different types of peri-aqueductal pneumatization: (1) large-cell pneumatization in the vicinity of the aqueduct; (2) small-cell pneumatization in the vicinity of the aqueduct; (3) absence of air cells. P = pneumatization; ae = external aperture of the vestibular aqueduct.



7 A B Different shapes of the external aperture of vestibular aqueduct (arrows with black outlines) to the paucity of air cells in the lower part of the pyramid and the mastoid portion of the left specimen (A) and a very small external aperture of the aqueduct, in

contrast to the extensive pneumatization of the pyramid and mastoid portion of the right specimen with a large external aperture of the aqueduct (B) MAI internal acoustic meatus SS sigmoid sulcus





Fig. 8 Plastic mould of a human inner ear demonstrating the course of the vestibular aqueduct (white arrow) and adjacent para-vestibular canaliculus (two arrows, white outlines) from the medial wall of the vestibule to the cerebellar surface of the pyramid. The bend of the vestibular aqueduct lies next to the tip of the white arrow. The para-vestibular canaliculus runs close to the posterior

semicircular canal (PSC). The upper facial nerve is marked with an asterisk. Note the minute vascular communications between the aqueduct and the para-vestibular canaliculus, which in its distal portion is split into branches (the arrow furthest to the left). AM, acoustic meatus; SSC, superior semicircular canal; LSC, lateral semicircular canal.

and no correlations were found (Table III). High age was not reflected by a non-visible vestibular aqueduct. This was surprising in view of the findings of Wittmaack (1956) and Zechner (1973) of bony obstruction of the aqueduct in elderly subjects, which might be expected to make the radiographic identification more difficult. It must be pointed out, that no very old persons were included in our material, where the age of the normal subjects ranged from 15–49 years (mean 33 years) and that of the patients with Menière's disease from 27–76 years (mean 48 years).

Besides differences in the aqueductal length between normal subjects and patients with Menière's disease, there are also obvious differences in the degree of pneumatization in these groups. Large cell pneumatization in the walls of the aqueduct is accompanied by long para-vestibular canaliculi. As patients with Menière's disease generally lack pneumatization of the pyramids, the aqueducts will be short. Presuming that pneumatization of the pyramids is regulated by genetic factors, it is possible that failure of pneumatization and subsequent shortness of the aqueducts predispose to Menière's disease.

has been found that the para-vestibular sinus, which is sometimes visible on tomograms of normal individuals as well as of patients with Meniere's disease, can be demonstrated by means of plastic moulds (Wilbrand & Stahle, 1974). The method will be described in detail in the forthcoming paper (Wilbrand, 1974). Generally, it runs adjacent to the vestibular aqueduct.

8) Distal branching occurs. Several minute anastomoses between the canaliculus and the aqueduct have been disclosed, housing small blood vessels. Their intimate topographical relationship to the vestibular aqueduct contrasts with the complexity of the morphological structures which with all probability are linked to the pathogenesis of Meniere's disease.

## ZUSAMMENFASSUNG

Aquaeductus vestibuli liess sich in Tomogrammen von normalen Personen immer darstellen. Bei Patienten, die schwerem Morbus Meniere leiden, ist die Darstellbarkeit beeinträchtigt. Der Aquädukt war bei einem gesunden Erkrankten im gesunden Ohr in 65%, und im kranken Ohr in 59% der Fälle sichtbar. Die entsprechende Seite bei doppelseitig Erkrankten war nur 53%. Die Krankheitsdauer hat einen Einfluss auf die Darstellbarkeit, welche jedoch in keinem Zusammenhang mit dem Lebensalter steht. Zwischen der tomographischen Darstellbarkeit und den Ergebnissen der Gehörprüfung und der kalorischen Probe wurde kein direkter Zusammenhang gefunden. Der Aquaeductus vestibuli ist bei Menierepatienten besser als bei gesunden Personen. Die Pneumatisation der Pyramide, welche bei Gesunden wie auch bei Menierepatienten gewisse Schwankungen aufweist, hat einen Einfluss auf die Länge des Aquäduktes. Die längsten Aquädukte wurden bei Gesunden gefunden, welche eine ausgedehnte Pneumatisation in der direkten Umgebung des Aquäduktes besaßen. Die Mehrzahl der Menierepatienten hatte kaum pneumatisierte Pyramiden, und die kürzeren Aquädukte wurden auch bei ihnen gefunden. Mit plastischen Gussmodellen ist der Aquaeductus vestibuli und ihn begleitende paravestibuläre Canaliculus abgebildet worden. Winzige Verbindungskanäle, die wahrscheinlich Teile der Blutversorgung des Ductus und des endolymphaticus beherbergen, sind veranschaulicht worden. Der paravestibuläre Canaliculus liegt im Grenzbereich zwischen dem darstellbaren und konnte in Tomogrammen von 32 Ohren gesunder Personen dargestellt werden, jedoch nur in 10 Fällen von 86 Ohren mit Menierekrankheit. Diese Struktur dürfte für den Abfluss des Saccus endolymphaticus von Bedeutung sein und damit einen Einfluss auf die Funktion des Innenohrs ausüben.

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## A THREE DIMENSIONAL MODEL OF THE CAT LABYRINTH

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(Received October 31, 1973)

**Abstract** A method is offered for the construction of a three dimensional plexiglas model of the cat vestibular organ

Knowledge of both the anatomy and orientation of the various structures comprising the vestibular end organ is essential for an understanding of the physiology of the vestibular system

A three dimensional plexiglas model of the labyrinth, magnified sufficiently, would aid in visualization of the vestibular apparatus. Through the use of plexiglas, anatomical relationships between superficial as well as interior structures can be examined. The purpose of this paper is to present the method for construction of this model.

### METHOD

Temporal bones of a cat were removed, fixed in formalin, and embedded in celloidin. Sections were cut at 20  $\mu$ m, mounted, and stained with hematoxylin and eosin. The bone was oriented for cutting to assure that the macula of the utricle was approximately in the horizontal plane. This ensured that the model when assembled would show the desired structures as they existed in the head erect position of the cat. The bone may be oriented in any other position, but this would cause the assembled model to be tilted in a position other than head erect.

Each fifth section was used for the construction of the model with the exception of those areas

where larger intervals were sufficient for the needed detail (i.e., from the level of the common crus to the apex of the vertical semicircular canals). A micro projector (Bausch & Lomb, triplex) was used to magnify the slide onto a clear plexiglas square measuring 6"  $\times$  6"  $\times$  1/8". A  $\times$ 20 magnification factor was determined from the ratio of the size of the anatomical structures on the plexiglas square to their size on the slide. This factor limited the thickness of the plexiglas sheet, as any thickness greater than the distance between corresponding surfaces of successive sections times the magnification factor would cause distortion of the three dimensionality of the model along this axis.

The bony and membranous labyrinths were traced onto each square using a LeRoy writing set with indelible ink of different colors. The cristae of the semicircular canals and maculae of the otolith organs were distinguished from other structures by a thicker inked line. Precise positioning of the projected image on each square was necessary for alignment of the desired structures in the assembled cube. The use of specific landmarks, such as mastoid air spaces, was helpful for this.

The model was assembled with two 1/4" bolts. The separation between the plexiglas squares was accomplished with tubular plexiglas spacers. These spacers were cut to a thickness that would make the distance between corresponding surfaces of each successive plexiglas square equal to the distance between the cor-

responding temporal bone sections times the magnification factor

In order to provide clarity, the picture (Fig. 1) was taken with the model submerged in water. Because the optical densities of water and plexiglas are similar, the three dimensionality of the model becomes evident. Knowing that a two dimensional picture does not allow for a three dimensional interpretation, the photograph was taken with a small depth of field hoping to give the impression of a third axis.

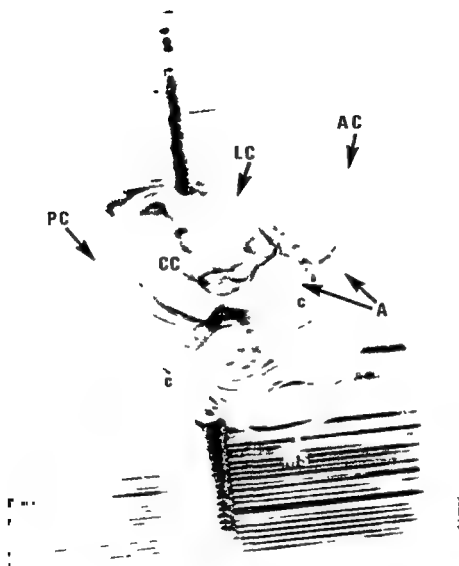
## ACKNOWLEDGEMENT

The technical assistance of the Temporal Bone Laboratory, Department of Surgery, Division of Otolaryngology, University of Florida, Gainesville, Florida, is appreciated.

## ZUSAMMENFASSUNG

Eine Methode zur Herstellung eines Plexiglasmodells des vestibulären Endorgans der Katze wird vorgelegt.

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*Fig 1* Three dimensional plexiglas model of the right labyrinth of a cat. Structures from lateral and inferior to medial and superior. The bony and membranous are shown in yellow and black respectively.

LC	lateral semicircular canal	MS	macula of the saccule
AC	anterior semicircular canal	CC	common crus
PC	posterior semicircular canal	A	ampulla
MU	macula of the utricle	c	cristae

(Photographic technique: see text.)



## EFFECTS OF D-TUBOCURARINE ON THE AMPULLAR RECEPTORS OF THE FROG

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(Received May 7 1973)

**Abstract** In order to better define the action of D-Tubocurarine on the ampullar receptors in frog vestibular apparatus the drug was applied to isolated open semicircular canals as well as to the entire undissected labyrinth. Curare was able to depress or abolish according to the doses employed (50-200  $\mu\text{g/ml}$ ) both the resting discharge of the ampullar receptors and their responses to mechanical or electrical stimulations only when it was administered to the isolated open semicircular canals. Contrariwise almost no effect was detectable when curare was administered to undissected preparations. The action of this drug develops earlier on the sensory units responsible for spikes of higher amplitude than on those responsible for medium and lower spikes: this effect may be interpreted as an enhancement of the threshold extended to the whole receptor population comprising the crista ampullaris. Curare blockade of the mechanical responsiveness in ampullar receptors is fully reversible: about 15 min after withdrawal of the drug while both resting discharge and electrical responsiveness are much more rapidly restored and even enhanced in excess of normal values after washing. The site and the possible mechanism of action of D-Tubocurarine on ampullar receptors are discussed.

Some observations indicate that curare is able to interfere in the mechanism of sensory processing taking place in labyrinthine receptors.

In preliminary experiments, Dohlman (1967) observed that curare can depress sensory discharge in semicircular canals. Kuiper (1956) found that the curare-like substance Flaxedil depresses the microphonic effect of the lateral line organ and he interpreted this action of the drug as being due to the impairment of a cholinergic process located in the mechano-sensitive membrane of the hair cells.

The presence of a cholinergic step in the activation of labyrinthine receptors is, however, a debatable point. On the basis of histochemical evidence Lowenstein (1967) and Vinnikov (1969) have suggested that acetylcholine might be the transmitter at the cytoneural junctions of ampullar receptors, this view has however been disproved by the data of Osborne & Thornhill (1972) and Thornhill (1972) which point to a monoamine as being the transmitter at these junctions.

On the other hand, Ireland & Farkashidy (1961) and Hilding & Wersall (1962) claim that acetylcholine may mediate the inhibition of ampullar receptors at the junctions of the efferent fibres.

The present experiments were devised with a view to a better definition of the action of D-Tubocurarine in the sensory apparatus of semicircular canals and to gain some insight into the processes which take place in the ampullar receptors.

Curare was applied to isolated open semicircular canals as well as to the entire undissected labyrinth of the frog. In the former condition the drug most probably contacts both poles of the sensory cells rapidly. In the second, curare very likely reaches the outer pole of the sensory cells with ease but its penetration into the endolymphatic space is likely to be hindered or even

This study was supported by the Grant No. 7100793/04 from the Italian National Research Council (C.N.R.)





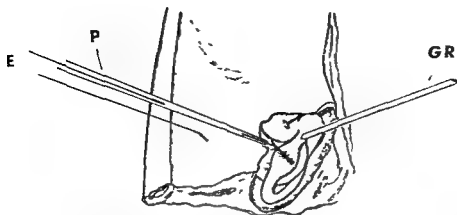


Fig 2 Schematic representation of the experimental set up for stimulating and recording of action potentials in

undissected preparations P, leading-off pipette, E, Ag/AgCl electrodes, GR, stimulating glass rod

labyrinth The ampullar nerve was carefully dissected

Mechanical stimulation of the ampullar receptors was performed by means of a thin glass rod placed against the wall of the canal near its opening into the utricle (Fig 2) The glass rod was driven by the moving coil of the small loud-speaker and its step displacements were so chosen as to evoke discharges similar to those produced in isolated preparations

In all the experiments the afferent discharge as picked up from fibre twigs dissected from the ampullar nerve, which were sucked into a glass pipette provided with Ag/AgCl electrodes.erve action potentials were amplified in a c 1000  $\times$ , monitored on a CRO and recorded on FM magnetic tape The spikes were counted electronically and classified into two groups by means of an amplitude discriminator, i.e.  $>150$   $\mu$ V (higher voltage spikes) and  $\leq 150$   $\mu$ V (medium and lower voltage spikes)

The responses of the ampullar receptors to mechanical and electrical stimuli were recorded before and during the exposure to curare for 15 min and also after withdrawal of the drug D-tubocurarine was added to the bath in such amounts as to reach final concentrations ranging between 50 and 200  $\mu$ g/ml

At least 5 similar experiments from different preparations were carried out in each experi-

mental condition Averaging and processing of data were performed by means of a CAT 400 C interfaced with a PDP 8/L computer

## RESULTS

Fig 3 shows an example of the tracings obtained from experiments in which D-Tubocurarine was applied to the intact labyrinth It may be noted that even at the higher doses tested (200  $\mu$ g/ml) the drug affected neither the resting discharge of the receptors nor their response to mechanical stimulation to any substantial degree

On the contrary, D-Tubocurarine produced a marked depression of the receptor discharges in isolated open canals Its action was already evident at a concentration of 50  $\mu$ g/ml and increased with the dose until, at 150–200  $\mu$ g/ml,

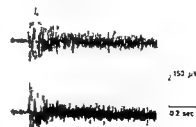


Fig 3 Discharges of ampullar receptors in response to mechanical stimulation in an undissected preparation Upper trace: control Lower trace: after exposure to D-Tubocurarine (200  $\mu$ g/ml) for 15 min

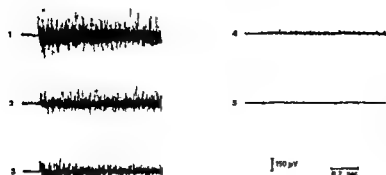


Fig 4 Discharges of ampullar nerve in response to a mechanical stimulus of medium strength recorded 10 min after exposure to D-Tubocurarine at different concentrations: 1 control, 2 3, 4 5, 50, 100, 150, 200  $\mu$ g/ml of Tubocurarine/ml of Tyrode solution.

both resting and evoked discharges were completely suppressed. The blockade by D-Tubocurarine develops in a few minutes and reaches a steady level in about 10–15 min.

As illustrated by the tracings in Fig 4, curare block becomes established in the receptors responsible for spikes of higher voltage even at the lowest dose employed, whereas a complete block of the receptors giving rise to spikes of medium and lower voltage was produced by D-Tubocurarine only at the highest doses (150–200  $\mu$ g/ml). However, after the action of moderate doses of the drug (Fig 5) the units which no longer responded to weak mechanical stimuli could be reactivated by increasing the stimulation strength, thus revealing that curare had enhanced the threshold of these units.

The dose-effect relationship may be deduced from the graphs in Fig 6 which refer to peak discharge frequency of the responses to mechanical stimulus of medium strength. Five rate graphs are drawn for the lower and red voltage (A) and the higher voltage spikes (B).

Fig 6C also illustrates the action of D-Tubocurarine on the resting discharge, which was a rule evaluated over the 30 sec preceding stimulation. It may be seen from the graph in figure that blockade of the resting discharge requires concentrations of the drug much higher than those able to depress the response of receptors to mechanical stimuli.

The effects of D-Tubocurarine are not reversed after washing. This is clearly evident in the graphs in Fig 6, which evidence a

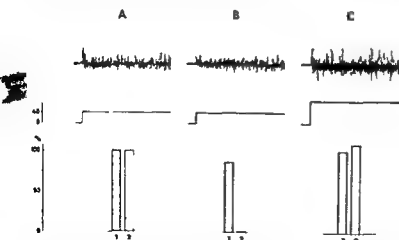
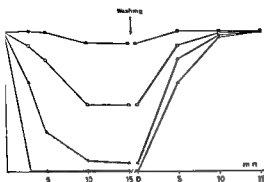


Fig 5 Responses to weak and to strong mechanical stimuli before and after exposure to a moderate dose of D-Tubocurarine (50  $\mu$ g/ml). (A) Response to the weak stimulus in plain Tyrode. (B) Response to the same stimulus 10 min after addition of D-Tubocurarine. (C) Response to a stimulus of doubled intensity 15 min after addition of D-Tubocurarine. Upper part: examples of the

records. Middle part: stimulus intensity expressed as placement of the microsyringe plunger. Lower part: histograms refer to the peak responses (mean  $\pm$  experiments) expressed as percentages of normal: medium lower voltage units (1) and for higher voltage units (2).

A



B

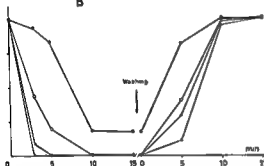
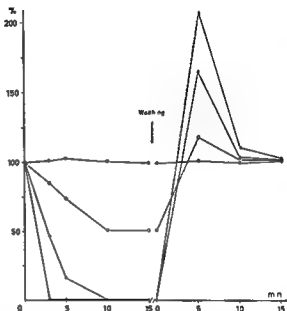


Fig. 6 Time course of the effects of D-Tubocurarine at different concentrations. Left side of the picture: peak responses to mechanical stimulation of medium intensity voltage units (A) and of the higher intensity units (B). Right side: mean frequency of the resting discharge evaluated before stimulation (C). Wash

C



ing at the break in the abscissae. The values are expressed as percentages of normal. Means from 5 experiments. ● D-Tubocurarine 50 µg/ml ○ D-Tubocurarine 100 µg/ml ▲ D-Tubocurarine 150 µg/ml △ D-Tubocurarine 200 µg/ml.

lete recovery of receptor responsiveness, 10–15 min after withdrawal of the drug, irrespective of its concentration. It may be noted (Fig. 6C) that the resting discharge recovers much earlier than the response to mechanical stimuli, being nearly complete within 2–3 min after washing. Furthermore, there is a period of 5–10 min, during which the resting discharge of the receptors is markedly enhanced to above its normal level; this enhancement is greater the larger the dose of curare, and therefore the deeper the depression previously suffered by the receptors.

Some examples of tracings from the experiments on open canals in which both mechanical and electrical stimuli were employed are reported in Fig. 7. A quantitative comparison between the responses to both electrical and mechanical stimulation as obtained from all

these experiments can be made from the graphs in Fig. 8. It can be seen that at a dose of curare capable of abolishing the responses to mechanical stimuli (200 µg/ml), D-Tubocurarine is also able to reduce considerably the receptor discharge evoked by electrical stimuli. The response to electrical stimulation, however, was not completely suppressed by the drug at the doses we used.

The comparison between the effects of each kind of stimulation during the recovery period following withdrawal of the drug appears to be of particular interest. In fact, it may be seen that the electrical excitability of the receptors becomes completely restored much earlier (2–3 min after washing) than their ability to respond to mechanical stimuli; furthermore, responses in excess of normal values to current flow are

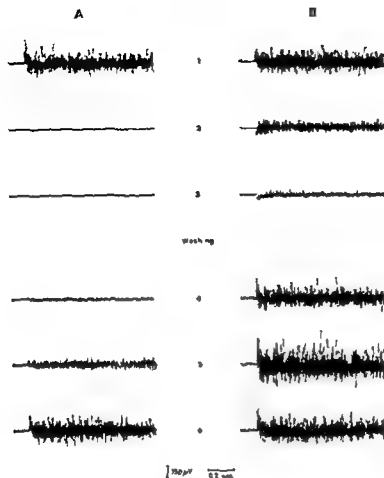


Fig 7 Comparison between the responses of the ampullar receptors to a mechanical (A) and to an electrical (B) stimulus of medium intensity. 1 controls; 2, 3, 10 min after D Tubocurarine 200 µg/ml; 4, 5, 6 1, 3 and 10 min after washing.

observed after recovery. This period of above normal responsiveness of the receptors to electrical stimulation was found to parallel the recovery of their resting discharge previously

described. The divergence between electrical and mechanical responsiveness reaches a maximum in the first few minutes after washing when the preparations appear to be completely insensitive to excitatory cupula deflection but give above normal discharges in response to electrical stimulation.

## DISCUSSION

The present experiments have clearly shown that curare can affect the impulse discharge of ampullary receptors only if it is allowed to reach the hair-bearing surface of sensory cells. Curare did in fact prove to be ineffective on the isolated labyrinth where its walls are likely structurally to hinder or even prevent penetration into the endolymphatic space.

In isolated preparations curare acts to partially or completely block both the



Fig 8 Time course of the peak responses of the ampullar receptors to mechanical (O) and to electrical (●) stimuli of medium intensity during the exposure to D Tubocurarine 200 µg/ml and after washing (at the break in the abscissae). The values are expressed as percentages of normal (means from 5 experiments).

large and the response of the ampullary receptors to mechanical and electrical stimuli accord to the dose employed. The depressant action of the drug appears to be established through a progressive increase in the receptor threshold. This mechanism may explain why curare block develops earlier in receptors responsible for peaks of higher amplitude since these receptors probably have a higher threshold in nature (Tagliati et al., 1974).

As to the actual mechanism of curare action, lack of any effect of D Tubocurarine on the intact labyrinth, where the drug is believed directly to contact the outer structures of the receptors, leads us to rule out any possibility of competitive interaction with the transmitter at the cytoneuronal junction of the ampullar receptors. It therefore seems more likely that curare may interfere with some step in the activation processes of the sensory cells which occur at the hair bearing pole. The experiments further have revealed that spontaneous activity and mechanical responsiveness of the receptors are not affected by the drug to the same degree. This is suggested by the differing time course between the recovery of the receptor resting charge and that of their responsiveness to mechanical stimuli, observed after withdrawal of the drug. During the recovery period following curare block, ampullar receptors did in fact were able to display a normal or even enhanced resting activity, whereas they were still completely insensitive to excitatory cupula deflections. In order to interpret this divergence the hypothesis may be put forward that two curare sensitive mechanisms to some extent mutually dependent may be present in the hair bearing membrane of the sensory cells. The first of these, which is more rapidly and steadily impaired by the drug, is likely to be involved in mechanoelectrical transduction, whereas the second, which is only affected by high doses of curare and is easily reversible, may support the resting charge in the receptors. Obviously, both mechanisms must ultimately follow the same pathway since they both lead to the release of chemical transmitter at the cytoneuronal junctions.

Insofar as confidence may be placed in the thesis that D Tubocurarine acts as a specific acetylcholine competitor, at the doses employed, the present results may indicate that some cholinergic step is involved in the processes which take place in the hair bearing membrane of the sensory cells. This view is in agreement with Kuiper's interpretation of the action of Flaxedil on the lateral line organ.

The depressant action of curare on electrical responsiveness of the ampullar receptors should be discussed in the light of the aforementioned factors.

Our experiments have shown that curare treatment prevents most of the excitatory effects produced by current flow through the crista ampullaris. This indicates that stimulation of the ampullar receptors by current flow involves the same processes which are blocked by curare and which are thought to be located in the hair-bearing membrane of the sensory cells. It follows that only the portion of the receptor response to electrical stimuli which is retained during curare block is likely to be due to direct action of the current on the endings of the afferent fibres (Löwenstein, 1955) and/or to an increased amount of chemical transmitter released at the cytoneuronal junctions (Del Castillo & Katz, 1954; Liley, 1956).

In this connection it is worth noting that during the clearing of curare block the recovery of the responses to electrical stimuli closely parallels that of spontaneous discharge. This similarity suggests that electrical stimulation of the ampullar receptors mainly activates the same mechanism which supports their resting discharge.

In order to interpret the enhancement to above normal values of the spontaneous discharge and electrical excitability in the receptors which are observed after washing of the drug, it may be supposed that during complete curare block, the transmitter accumulates inside the sensory cells and that this accumulation facilitates release of the transmitter after withdrawal of the block.

On the basis of the present experiments, the

structures in which the processes underlying resting activity and mechano-sensitivity of the ampullar receptors take place, cannot be positively identified. The action of curare did reveal, however, that both processes are located in the hair-bearing pole of the sensory cells. Kinocilium and stereocilia are therefore most likely to be the structures involved.

## ACKNOWLEDGEMENT

We are much indebted to Professor C. Casella, Head of the Institute of General Physiology of Pavia, for his generous help and valuable suggestions in designing the experiments and in discussing the results.

## ZUSAMMENFASSUNG

Zur besseren Bestimmung des Effekts von D-Tubocurarin bei Ampullarrezeptoren im Vestibularapparat des Frosches wurde die Substanz sowohl den isolierten semizirkularen Kanälen als auch den nichtisolierten Labyrinthin verabreicht. Nur bei Anwendung in den isolierten semizirkularen Kanälen konnte das Curare je nach der angewandten Dosis (50–200 µg/ml) sowohl die Ruheaktivität der Ampullarrezeptoren als auch ihre Erwidern auf die mechanische und elektrische Stimulation vermindern oder beseitigen. Sobald das Curare den nicht isolierten Präparaten verabreicht wurde, war hingegen fast kein Effekt feststellbar. Die Aktion des Curare tritt bei jenen Rezeptoren, die für das Aktionspotential mit höheren Amplituden verantwortlich sind, früher auf als bei den für das mittlere und niedrigere Aktionspotential verantwortlichen. Dieser Effekt kann als eine Erhöhung der Schwelle ausgelegt werden, die sich auf die Gesamtheit der die Crista Ampullaris bildenden Rezeptoren auswirkt. Die Curare Blockierung der mechanischen Erwidern in den Ampullarrezeptoren ist in etwa 15 Minuten nach Entzug der Substanz vollkommen reversibel, während sowohl die Ruheaktivität als auch die elektrische Erwidern sich wesentlich rascher normalisieren und nach der Spülung sogar die Normalwerte übersteigen. Die Lage und der mögliche Effektmechanismus des D-Tubocurarin in Ampullarrezeptoren wurden diskutiert.

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## MENIÈRE'S DISEASE

### *Preliminary Report of Lithium Treatment*

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(Received November 12, 1973)

**Abstract** On the assumption that the hydrops of the lymphatic space present in Menière's disease is due to defective transport of water and ions between the sub- and perilymphatic space the authors in an open study treated patients with this disease with lithium carbonate as lithium among many biological effects acts on transport of ions across membranes. Our results showed that in 70% (21 patients) the effect of the treatment was excellent, judging by the patients' own state of affairs concerning the reduction in the frequency and intensity of the attacks. The remaining 30% (9 patients) gained no relief or only a doubtful effect of the treatment. Although a placebo effect cannot be ruled out in this open trial the high (70%) and persisting (17 months) effect does justify continued investigations into the effect of lithium upon Menière's disease.

Enough Menière's disease is clinically well defined, very little is known as yet about its etiology and pathogenesis despite several investigations into the anatomical, physiological, and biochemical changes.

Nevertheless, studies on temporal bones of patients with Menière's disease have disclosed marked dilatation of the endolymphatic space, especially the cochlear duct, saccule, and utricle (Luknecht, 1968). This so-called hydrops has been confirmed at operation (Portmann, 1973). The hypotheses advanced concerning the cause of this hydrops have mainly been centred on two mechanisms. Firstly, the vascular genesis which tries to explain the changes by episodic spasms or thromboses in the arterial blood

supply of the inner ear (Lawrence, 1968). Secondly, the neurogenous theory, holding that changes in the tonus of the efferent nerve supply to the inner ear affects the ability of the labyrinthine membranes to maintain the high concentration of potassium in the endolymphatic space (Williams, 1965).

The pharmacological treatment of the disease has, therefore, been mainly channelled in two directions, one aimed at affecting the vascular system by vasodilating agents and the other towards the central and peripheral neurochemical mechanisms by means of sedatives, ganglion blockers, sympathocolytics, antihistaminics, etc.—with varying success, according to the various sources (Altmann, 1955; Meyer, 1966).

A third hypothesis is that Menière's disease is a local manifestation of a systemic disturbance in the regulation of the ratio between extracellular and intracellular fluid. Therapeutic attempts have therefore been made with a diet low in fluid and salt as well as with diuretics, but with varying and doubtful results (Mygind & Dederding, 1938; Altmann, 1955).

However, it might be reasonable to look for a medication which could influence the transport of water and ions into and out of special biological compartments such as, for instance, the endolymphatic space. Lithium might be a drug having these properties (Rafacelsen et al.,



1973, Singer & Rotenberg, 1973) Lithium is used in psychiatry for the treatment of manic-depressive psychosis. Its effect upon mania is well documented (Schou et al., 1954), whereas that upon depression is still a moot point (Goodwin et al., 1969). The main indication is, however, its dependable and still unique prophylactic action both on manic and depressive states (Baastrup et al., 1970).

Lithium is administered in tablets containing 300 mg lithium carbonate. The daily dose is 600–1 800 mg (16–48 milliequivalents) given in a single dose in the evening, aiming at a lithium concentration of 0.7–1.3 mmol/l in the serum drawn 12 hours after ingestion of the tablets.

Correctly administered lithium medication gives but few unwanted effects: (1) Tremor which responds to treatment with propranolol (Kirk et al., 1972), (2) polyuria with secondary polydipsia, (3) weight gain, and (4) goitre, which is usually euthyroid, but may be hypothyroid. At serum lithium levels exceeding 1.3 mmol/l most patients develop incipient toxic symptoms in the form of nausea and vomiting, at times accompanied by diarrhoea, more marked tremor, as well as fatigue and drowsiness. At even higher serum levels (above 2.0 mmol/l) these symptoms will be intensified and cerebral symptoms of various kinds will occur. Severe poisoning may be fatal. Although the balance between therapeutic and toxic serum concentrations of lithium is finely poised, experience has shown that lithium medication under careful supervision is a justified and safe treatment.

Numerous investigations have been carried out with a view to elucidating the mechanism of lithium's effect in the hope that this would clarify the biochemical basis of manic-depressive psychosis (Rafaelson & Møllerup, 1973; Schou, 1973). However, it must be admitted that the mode of action of lithium in this disease is still poorly understood.

Lithium is predominantly used in psychiatry, but preliminary findings indicate that it may be of value also in thyrotoxicosis (Spaulding et al., 1972) and in Huntington's chorea (Dalen, 1973; Mattsson, 1973).

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## MATERIAL AND METHODS

### Subjects

Forty out-patients with Meniere's disease investigated. All patients fulfilled the following five subjective criteria: (1) hearing impairment, (2) tinnitus, (3) vertigo, (4) roaring, and (5) vomiting. Objectively, all patients had perceptible hearing loss and recruitment, measured by the method of Fowler and co-workers. Only patients with normal renal and cardiac functions checked by normal serum creatinine and absence of proteinuria, normal ECG and chest radiography, were included.

The duration of the symptoms averaged 10 years, with a wide range—2 months to 20 years. The frequency of the attacks was described as follows: 16 patients had daily attacks, 13 patients had about one attack weekly, and 11 patients had one attack monthly.

### Treatment

All the patients had previously been treated on an out-patient basis by otological practitioners with antihistaminics, sedatives, and vasodilators, with little or no effect.

At the commencement of treatment all patients were informed about the possible effects of lithium and the signs of poisoning. The initial dose was 600–900 mg lithium carbonate, maintenance dose 600–1 200 mg daily. It was endeavoured to keep the serum lithium concentration in the range 0.7–1.3 mmol/l. The concentration was checked weekly in the first month, the next 6 months it was checked monthly, and thereafter every other month. Serum lithium was determined by flame photometry (Amdisen, 1967).

### Therapeutic efficacy

The patients' own statements about the frequency and severity of attacks were used in estimating the therapeutic efficacy.

## RESULTS

On the basis of the therapeutic effect the material is divided into 3 groups (Table 1). C

Table I Serum lithium concentrations, duration of treatment and of Meniere's disease in 40 patients

Categories	No. of patients	Average serum lithium concentration (range all values) (mmol/l)	Average duration of treatment (range) (months)	Average duration of disease (range) (years)
<b>Group I</b>				
Under treatment with good effect	16	0.7 (0.3-0.9)	17 (3-30)	5 (1-15)
Treatment discontinued because of success of treatment	5	0.7 (0.4-0.9)	14 (10-18)	
<b>Group II</b>				
Treatment discontinued because of lacking effect	9	0.6 (0.3-0.8)	4 (1-12)	10 (1-20)
<b>Group III</b>				
Treatment discontinued because of side effects	10	0.6 (0.4-1.0)	2 weeks (5-16 days)	5 (1-20)

consists of 21 patients in whom the therapeutic result was called excellent. Sixteen of these patients were still on lithium, which they have been taking for an average of 17 months (range 3-30 months). These patients have no symptoms of their Meniere's disease, and only a few unwanted effects. These effects were few, relatively mild, and have not affected the patients' ability to continue the treatment. Another 5 patients interrupted the treatment of their own accord after an average of 14 months, as they felt perfectly well.

Group II is comprised of 9 patients who did not respond properly to lithium therapy. These patients took lithium for an average of 4 months (range 1-12 months).

Group III is comprised of 10 patients in whom treatment was discontinued because of unwanted effects. These effects occurred after medication in an average of one week, so that the patients received lithium for about 2 weeks only.

The therapeutic effect became manifest in most patients after 2-4 weeks of treatment.

From Table I it is apparent that the mean level of lithium was 0.7 mmol/l (range of values 0.3-1.0 mmol/l). With respect to serum lithium the three groups did not differ from each other.

No serum lithium level was above the therapeutic level, and indeed we have had no cases of toxic symptoms.

As shown in Table I the average duration of the disease in group I was 5 years, and in group II 10 years. This difference between the two groups was statistically significant (the Mann-Whitney test, Siegel, 1956,  $P < 0.05$ ).

In Table II the frequency of the attacks shows, for group I, daily or weekly, 17, and monthly, 4; in group II, daily or weekly, 4, and monthly, 5. Using Fischer's exact probability test (Siegel, 1956) we found that group I had statistically significantly more often daily or weekly attacks ( $P = 0.06$ ).

The unwanted effects (Table III) were like those usually reported (Schou et al., 1971) but the severity was extremely varied. Tremor of the hands was by far the most common effect, but the medication was never withdrawn for this reason, as the tremor could be effectively controlled by propranolol 10 mg 3 times daily. The tremor appeared after only a few days' medication. Four patients out of 21 in Group I and one of the 9 in group II were treated with propranolol. With the exception of insomnia, weight gain in one case, and fatigue in another, the other unwanted effects appeared soon.

Table II *Distribution of frequency of attacks in 40 patients with Meniere's disease, treated with lithium*

Patient categories	No of patients with daily or weekly attacks	No of patients with monthly attacks	Total
<i>Group I</i>			
Good effect of lithium treatment	17	4	21
<i>Group II</i>			
Lacking effect of lithium treatment	4	5	9
<i>Group III</i>			
Patients with unwanted effects of lithium treatment	6	4	10

about one week of medication, and all gave occasion to discontinue the medication. The effect of lithium upon Meniere's disease is illustrated by the following case history in which the patient continued the medication in spite of severe unwanted effects.

#### Case History

A 52 year-old housewife referred to the University ENT Department, Rigshospitalet, by an otological practitioner with a diagnosis of Meniere's disease. There was no familial predisposition, especially not to manic-depressive psychosis.

Table III *Unwanted effects in 40 patients with Meniere's disease treated with lithium*

In order of frequency	No of patients
Tremor	10
Aggravation of symptoms	4
Fatigue	3
Nausea	3
Weight increase	2
Thirst and polyuria	1
Gastritis	1
Gout and myxedema	1
Edemas	1
Precordial pressure	1
Insomnia	1

For 8 years she had been developing increasing hearing loss in the left ear. During the same period frequent attacks of sudden vertigo was accompanied by tinnitus and vomiting. She had previously been treated with cinnarizine (an antihistamine) with no effect and with thietilperazine (a thiazine) with some effect upon the vertigo but not upon the frequency of the attacks. The perceptible hearing loss was 65 dB and a pronounced recruitment measured by the Hughson and Fowler methods, and left sided canal.

The patient was placed on lithium in tablet form, 900 mg/24 hours. The serum determinations were constant at 0.8 mmol/L. After about 3 weeks on this medication the patient obtained a marked improvement, in that the attacks had stopped. She had no effects of lithium in the form of tremor, hands, fatigue, and nausea. She was then treated with propranolol 10 mg 3 times daily with a good effect. Thereafter the patient was satisfactory and stable for 6-7 years. It was then noted that she had had a weight increase of 15 kilograms. The weight stabilized, and after 10 months' treatment she wanted to go on taking lithium, despite the marked weight gain, some tremor, and nausea, and as now she has only negligible complaints.

#### DISCUSSION

As already mentioned the therapeutic effect of lithium was manifest after 2 to 4 weeks on treatment. Therefore, the 10 patients in whom lithium was discontinued after only 2 weeks have been excluded from the assessment. Thus, 30 patients of whom 21 (70%) reported a subjective result of the treatment, judged as a reduction in the frequency and severity of attacks, whereas 9 patients (30%) had no effect or a doubtful effect.

The anamnesis of the remaining 29 patients showed that the group with a good therapeutic outcome had statistically significantly fewer daily or weekly attacks and a statistically significant shorter duration of the disease.

with a poor therapeutic outcome. Where the difference between the two groups in the frequency of attacks is difficult for us to explain, the finding that lithium is more apt to be effective in patients with a short history agrees with the findings that the changes of the inner ear in Meniere's disease initially are reversible and some years become permanent. If our assumption that lithium influences the transport of water and electrolytes into the endolymphatic space is correct, it must be expected that this can act only if some function of the membrane transport mechanisms remains, i.e., in cases with a short duration of the disease. We can only with reservations compare our preliminary results with other well substantiated clinical treatments of Meniere's disease, because our follow up period is short. McNally (1953) demands a 5-year observation period and preferably 5 years' freedom from symptoms, in assessing the results of medical treatment of Meniere's disease. Moreover, the assessment should be based upon double-blind studies without or with cross over design. As far as we know, there have been only three double-blind trials, viz., Arner et al.'s (1958), Philippon (1962), and Collins & Poe's (1962). These authors have studied the effect of antihistaminics and amphetamine upon Meniere's disease, and three groups found a statistically significant effect. Our lithium therapy was run as an open trial without all its inborn risks of erroneous conclusions. Although the good therapeutic result (70%) in cases that usually found with placebo, and although the effect has been maintained, for an average of 17 months, it cannot be denied that many very patients have been good placebo responders. With a view to the dosage and serum concentration of lithium we have endeavoured to place the patients within the range 0.7–1.3 mmol/l. This empirically has been found necessary in the treatment of manic-depressive psychoses. However, we do not know whether the same concentrations are needed, as we have long been inclined to keep one patient symptom free on a

serum lithium concentration of only 0.3 mmol/l. This patient, therefore, is not included in the analysis.

The unwanted effects of the lithium therapy correspond to those reported from the lithium therapy of manic depressive psychosis (Schou et al., 1971). It is remarkable, however, that the patient who developed goitre showed this effect after only 2 weeks' medication. We found a serum thyroxine level of 45 nmol/l (normal range 66–139 nmol/l), but there is no possibility of deciding whether the patient was hypothyroid prior to the treatment or whether the low serum thyroxine was induced by lithium.

These preliminary findings may indicate that treatment with lithium has a favourable effect upon the symptoms in patients with Meniere's disease. The results, moreover, justify further investigations, and in a joint project the University ENT Department and the Psychochemistry Institute, Rigshospitalet, have started a double-blind cross-over trial to confirm or disprove these preliminary results.

## ZUSAMMENFASSUNG

Es wird angenommen dass der bei der Menièr'schen Krankheit charakteristische endolymphatische Hydrops auf einer abnormen Förderung von Wasser und Ionen zwischen den endolymphatischen und den perilymphatischen Räumen beruht. Da Lithiumkarbonat unter vielen anderen biologischen Eigenschaften auch die Ionenwanderung durch Membranen beeinflusst, wurden Patienten mit der Menièr'schen Krankheit in einer offenen Pilot Study mit diesem Präparat behandelt. Ergebnis: 70% der Patienten (21) zeigten nach eigenen Beobachtungen ausgezeichnete Behandlungsergebnisse in bezug auf die Reduktion von Häufigkeit und Stärke der Anfälle. Bei den übrigen 30% (9) zeigte sich nur zweifelhafte oder gar keine Wirkung. Obwohl in dieser offenen Behandlung eine Placebo-Wirkung nicht ausgeschlossen werden kann, berechtigt der Anteil positiver Ergebnisse (70%) und zugleich deren Dauer (17 Monate) zu weiterer Untersuchung der Einwirkung von Lithium auf Patienten mit der Menièr'schen Krankheit.

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## ÜBER DIE VISKOSITÄT DER LYPHE IM INNENOH DES HAUSSCHWEINES

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(Eingegangen am 21. Januar 1974)

**Zusammenfassung:** Die Viskosität von Lymphe wird nach verschiedenen Methoden, die insbesondere bezüglich der notwendigen Korrekturfaktoren diskutiert werden, bestimmt. Kapillarmessungen können nur Meßergebnisse auswertet werden, die oberhalb eines Grenzdruckes gewonnen werden. Danach haben Peri- und Endolympe gleiche, etwa um 10% größere Viskosität als Wasser, doch eine um den Faktor 1,6 kleinere Viskosität als Serum.

### 1 EINLEITUNG

In den letzten Jahren wurde damit begonnen, hydromechanischen Vorgänge im Innenohr nicht nur für das System scala vestibuli-scala media-tympani zu untersuchen, sondern auch innerhalb der scala media (Helle, 1974; Helle, 1973; Zwicker, 1971, 1972, 1974). Neut hat bereits 1950 in Betracht gezogen, daß

Erregung der Sinneszellen mit Strömungen der scala media zusammenhängen könnte. Nach v. Illberg (1968) muß angenommen werden, daß die Endolympe nicht nur den sulcus sacculus, sondern auch den Spalt zwischen Deckmembran und lamina reticularis erfüllt. Wenn dies, wie an anderer Stelle diskutiert (Helle, 1974; Zwicker, 1972, 1974) Strömungen in diesem Spalt für die Verbiegung der Haare und damit für die Erregung der Haarzellen maßgebend wären, dann käme der Viskosität der Endolympe eine für die Beschreibung der ablaufenden Vorgänge entscheidende Bedeutung zu. Verschiedene Angaben über die Viskosität der Endolympe und der Perilymphe sind von Schnieder & Schindler (1964) gesammelt dar-

gestellt und durch eigene Meßergebnisse ergänzt worden. Letztere, sowie diejenigen von Money et al (1966) zeigen Viskositäten in der Nähe derjenigen von Wasser. Die Angaben anderer Autoren (Schnieder & Schindler, 1964) weichen um Faktoren davon ab. Es erscheint daher sinnvoll, die Ergebnisse nachzuprüfen und nach möglichen Gründen für Abweichungen zu suchen.

### 2 MESSMETHODEN

#### a) Ostwald Viskosimeter

Bei ihrem Gebrauch wird eine bestimmte Flüssigkeitsmenge durch die von ihrem Eigengewicht hervorgerufene Kraft durch eine senkrecht stehende Kapillare definierter Länge hindurchgedrückt. Sie sind im Handel erhältlich und sehr einfach in der Handhabung (Kohlrausch, 1968). Sie sind jedoch nur für Flüssigkeitsmengen von mindestens etwa  $1 \text{ cm}^3$  verwendbar. Die Peri- und Endolymphmengen, die einem Innenohr entnommen werden können, liegen jedoch in der Größenordnung  $\text{mm}^3$ , so daß das Ostwald-Viskosimeter für die Messung der Viskosität von Lymphe nicht in Frage kommt. Für Vergleichsmessungen an Eichflüssigkeiten ist es wegen seiner großen Genauigkeit sehr geeignet und wurde dazu auch benutzt.

#### b) Kapillarkiskosimeter

Sie bestehen aus horizontal liegenden Kapillaren mit kleinem Innendurchmesser  $\phi_x$ , in denen

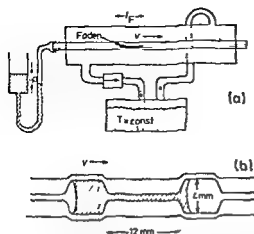


Abb 1 (a) Schematischer Aufbau zur Messung der Viskosität mit Hilfe der Verschiebungsgeschwindigkeit  $v$  eines Flüssigkeitsfadens der Länge  $l_F$  in einer Kapillare infolge der Druckdifferenz  $\Delta p$  (b) An zwei Stellen zu Zylindern aufgeweitete Kapillare. Bei diesem als „Miniviskosimeter“ bezeichneten Aufbau wird die Flüssigkeitsmenge (etwa  $20 \mu\text{m}^3$ ) durch  $\Delta p$  an den beiden Marken (gestrichelt) vorbeigeschoben und  $v$  bestimmt

durch äußere Druckdifferenz  $\Delta p$  ein Flüssigkeitsfaden der Länge  $l_F$  verschoben wird. Dem Hagenpoiseuille'schen Gesetz entsprechend gilt (Kohlrausch, 1968) folgender Zusammenhang

$$\Delta p = \frac{32\eta \cdot l_F \cdot v}{\varnothing_K^4} \quad (1)$$

aus dem sich die Viskosität  $\eta$  mit der Verschiebungsgeschwindigkeit  $v$  des Flüssigkeitsfadens rechnen läßt. Für eine Flüssigkeitsmenge von  $\text{m}^3$  ergibt sich bei  $\varnothing_K = 0,35 \text{ mm}$  eine Fadenlänge von etwa  $1 \text{ cm}$ . Weil während der Verschiebung des Flüssigkeitsfadens an seinem Ende immer etwas Flüssigkeit an den Kapillarinneiwänden zurückbleibt, ändert sich die Fadenlänge während der Messung ein wenig. Damit dieser Einfluß gering bleibt, sollten Fadenlängen unter  $1 \text{ cm}$  nicht benutzt werden. Da die Viskosität stark von der Temperatur abhängig ist, wurde die Kapillare (Abb 1a) in eine weitere Glasröhre so eingebaut, daß sie von Wasser konstanter Temperatur umspült werden konnte. Der Körpertemperatur des Hausschweines entsprechend wurden alle Untersuchungen bei  $39^\circ\text{C}$  durchgeführt. Die Druckdifferenz  $\Delta p$  zwischen den Enden der  $40 \text{ cm}$  langen Kapillare wurde

durch ein kommunizierendes Röhren-Schlauchsystem eingestellt und in cm Wassersäule (Ws) abgelesen.

Bei Flüssigkeitsfadenlängen, die die ganze Kapillarlänge erreichen, ist die Druckdifferenz, die zwischen den Kapillarenden herrscht, nicht gleich der Druckdifferenz zwischen den beiden Enden des Flüssigkeitsfadens wirkt, weil ein Teil der ersteren über die Luftsäule in der Kapillare abfällt, die verschoben werden muß. Demnach ist die Gesamtdruckdifferenz  $\Delta p$  aufgeteilt in die Druckanteile  $\Delta p_L$  und  $\Delta p_F$ , die zur Verschiebung der Luftsäule bzw. des Flüssigkeitsfadens notwendig sind.

Dabei gilt  $\Delta p = \Delta p_L + \Delta p_F$ .

Für die Auswertung wichtig ist lediglich die Druckdifferenz  $\Delta p_F$ , die über einen Korrekturfaktor  $k$  aus  $\Delta p$  bestimmt werden kann. (1) und (2) ergibt sich

$$k = \frac{\Delta p_F}{\Delta p} = 1 - \frac{1}{1 + \frac{\eta_L \cdot l_F}{\eta_F \cdot l_L}}$$

Allerdings muß die Viskosität  $\eta_L$  der Flüssigkeit bekannt sein, um die Korrektur durchführen zu können. In Tabelle I sind die Korrekturfaktoren  $k$  für eine Kapillarenessenz von  $40 \text{ cm}$  sowie für das benutzte „Miniviskosimeter“ (siehe unten) angegeben.

Eine Abschätzung der Viskositäten  $\eta$  wurde durch vorgenommen werden, daß  $\eta = f(\Delta p)$  die für eine Verschiebung des F

Tabelle I Korrekturfaktoren  $k_{H_2O}$  und  $k$  Wasser und Eichflüssigkeit für verschiedene Flüssigkeitsfadenlängen bei  $40 \text{ cm}$  Gesamtlängere und für das „Miniviskosimeter“

	$l_F [\text{cm}]$	$k_{H_2O}$	$k$
Kapillare $40 \text{ cm}$ lang	1	0,47	0,47
	3	0,74	0,74
	5	0,83	0,83
Miniviskosimeter		0,85	0,85

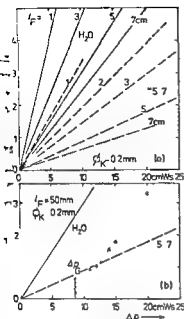


Abb 2 Verschiebungsgeschwindigkeit  $v$  als Funktion von  $\Delta p$  bei einem Kapillardurchmesser  $\sigma_K = 0.2 \text{ mm}$  (a) für Flüssigkeiten  $\text{H}_2\text{O}$  (durchgezogen) und 5 7 (gestrichelt) mit der Flüssigkeitsfadenlänge  $l_F$  als Parameter (b) Endolymphe ( $\Delta$ ) und Perilymphe ( $\circ$ ) im Vergleich mit den Ergebnissen aus (a) für  $l_F = 5 \text{ cm}$ .  $\Delta p_{90}$  schneidet den bei Lymphe auftretenden Grenzdruck

itsfadens um  $\Delta l$  (in unserem Fall  $\Delta l = 20 \text{ cm}$ ) notwendige Zeit als Funktion der Fadenlänge mit  $\Delta p$  als Parameter aufgetragen wird. Alle Kurven müssen Geraden sein, die sich auf der negativen Achse bei  $l_{FL}^*$  in einem einzigen Punkt

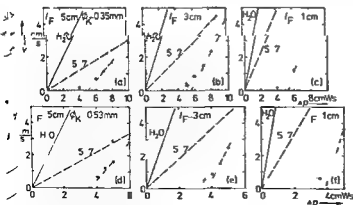


Abb 3 Wie Abb 2 (b) jedoch mit  $\sigma_K = 0.35 \text{ mm}$  und in Parameter  $l_F = 5 \text{ cm}$  (a)  $l_F = 3 \text{ cm}$  (b)  $l_F = 1 \text{ cm}$  (c)  $\sigma_K = 0.53 \text{ mm}$  und den Parameter  $l_F = 5 \text{ cm}$  (d)

schneiden. Aus (1) und (2) ergibt sich mit  $\eta = \Delta l / t$  und einer Konstanten  $A$

$$t \sim A \frac{\eta_L l_{FL} + \eta_z l_{Fz}}{\Delta p} \quad (4)$$

Wenn  $l_{Fz} < l_{FL}$  kann  $l_{FL}$  als konstant und die Gleichung

$$t = \frac{A}{\Delta p} \eta_z l_{Fz} + \frac{A}{\Delta p} \eta_L l_{FL} \quad (5)$$

als eine Schar von Geraden mit dem Parameter  $\Delta p$  angesehen werden, die sich im Punkt

$$l_{Fz}^* = -\frac{\eta_L}{\eta_z} l_{FL} \quad (6)$$

schneiden. (Für die mit  $\sigma_K = 0.35 \text{ mm}$  gewonnenen Ergebnisse (Abb 3, oben) ist die entsprechende Umzeichnung in Abb 4 für die Flüssigkeiten  $\text{H}_2\text{O}$  und 5 7 durchgeführt.) Die Viskosität von Luft hat bei  $39^\circ\text{C}$  den Wert  $\eta_L = 0.019 \text{ cp}$ . Damit läßt sich aus der umgeschriebenen Gl (6)  $\eta_z$  abschätzen

$$\eta_z = -\frac{l_{FL}}{l_{Fz}^*} \eta_L \quad (7)$$

Für die Bestimmung des Korrekturfaktors ist diese Abschätzung die meist eine Genauigkeit von 20% besitzt, ausreichend vorteilhaft ist ferner, daß Fehlmessungen, z. B. durch Verun-

$l_F = 3 \text{ cm}$  (e)  $l_F = 1 \text{ cm}$  (f). Außer für Perilymphe (+) sind auch Meßwerte für Blutserum (O) eingetragen.



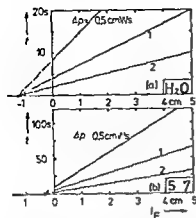


Abb 4 Für eine Verschiebung des Flüssigkeitsfadens um 20 cm notwendige Durchlaufzeit  $t$  als Funktion der Fadenlänge  $l_F$  für die Flüssigkeiten  $H_2O$  (a) bzw. 5:7 (b). Parameter ist die Druckdifferenz  $\Delta p$  (Methode 2b)

reinigungen hervorgerufen, in Darstellungen entsprechend Fig 4 sofort erkannt und eliminiert werden können

### c) „Miniviskosimeter“

Diese Art von Viskosimeter ist eine Kombination der Methoden a und b. Es benötigt eine Flüssigkeitsmenge von nur etwa 20 mm<sup>3</sup>. Eine Kapillare mit einem Durchmesser von 0,53 mm wurde an zwei Stellen im Abstand von 20 mm so aufgeblasen, daß zwei etwa zylindrisch geformte Behälter mit einem Innendurchmesser

4 mm entstanden (Abb 1b). Nachdem die Kapillare eingefüllt ist, wird sie wie bei Methode 2b durch die Druckdifferenz  $\Delta p$  in Bewegung gesetzt. Die Verschiebungsgeschwindigkeit wird aus der Zeitdauer  $t$  bestimmt, welche die Flüssigkeit benötigt, um mit ihrem linken Rand von der einen zur anderen Markierung (gestrichelt in Abb 1b eingezeichnet) zu gelangen. Da die Druckdifferenz auf verhältnismäßig großen Flächen angreift und demnach wesentlich größere Kräfte erzeugt als bei Methode 2b, stören kleine Druckänderungen, die im Röhren-Schlauch-System bei Temperaturschwankungen entstehen. Deswegen wurde vor jeder Druckeinstellung der 0-Punkt ( $\Delta p = 0$ ) durch Öffnung eines Abzweighahnes kontrolliert.

## 3 DIE ENTNAHME DER LYMPHE

Die Felsenbeine frisch geschlachteter Ferkel wurden im Schlachthof ausgenommen (Zwicker, 1971). Zur Entnahme von Endolymph wurde unter dem Operationsmikroskop das Innenohr nahe dem Helicotrema eröffnet (vestibuli) und zunächst etwas Perilymph genommen, so daß der Flüssigkeitspegel der Reissnermembran freigab. Mit einer feinen Nadel wurde danach die Reissnermembran durchstoßen und ein Teil der Endolymph abgeaspiert. In einigen Kontrollexperimenten wurde eventueller Durchbruch der Reissnermembran an anderer Stelle dadurch kontrolliert, daß bei der Entnahme von Endolymph die Perilymph im Durchspülverfahren durch eine andere Flüssigkeit ersetzt wurde. War die Endolymph ohne Anfärbung, so war die Reissnermembran nicht gebrochen und die Endolymph reiner Perilymph vermischt. Durchbrüche traten auf, wenn zuviel Endolymph abgesaugt wurde.

Perilymph wurde entweder durch das Fenster oder/und am Helicotrema entnommen. Sie war einfacher und auch in größerer Menge zu gewinnen als Endolymph.

Die Präparation der Lymphflüssigkeit meist nach einer Stunde post mortem bei 4°C. Wenn nicht anders angegeben, wurden die Flüssigkeiten innerhalb der zweiten Stunde durchgeführt, die Lymphe demnach nicht länger als 2 Stunden post mortem benutzt. Fast jede Versuchsserie wurde mit Lymphe von einem anderen Tier durchgeführt. Weil sich dabei keine signifikanten Unterschiede ergaben, wurde Lymphe auch aus verschiedenen Ohren gesammelt. Daß mit größeren Fadenlängen (Methode 2b) und mit dem „Miniviskosimeter“ gearbeitet werden konnte.

## 4 ERGEBNISSE

Neben Endolymph und Perilymph wurde auch zum Zwecke des Vergleichens und des Einflusses von reinem Wasser und einer Eichflüssigkeit (Eichflüssigkeit diente eine Mischung

Tabelle II Die nach den beschriebenen Methoden gewonnenen Viskositäten Temperaturen und Höhen sind ebenfalls angegeben

Flüssigkeit	Temp [°C]	rel Dichte	Viskosität			
			Nach 2a abs [cP]	rel	Mittelwerte nach 2b rel	Nach 2c rel
Wasser	20		1,002			
	39	1	0,659	1	1	1
Blutserum (Hausschwein)	39	1,035	1,13	1,73	1,72	1,69
7	39	1,37	2,52	3,82	3,80	3,70
Lymphe ungefiltert	39				1,17	1,20
Lymphe gefiltert	39					1,09

Chromschwefelsäure<sup>2</sup> und dest Wasser (H<sub>2</sub>O)

■ Volumverhältnis 5/7, die im Folgenden als 5/7 bezeichnet ist. Nachdem sich eigenartige Abhängigkeiten der Verschiebungsgeschwindigkeit vom Druck sowohl für die Peri- als auch für Endolympe ergaben (siehe unten), wurde Blutserum (Hausschwein, 2 mal 20 min bei 100 Umdr./min zentrifugiert) mit in die Meßreihen einbezogen, weil es nicht nur ähnliche Effekte zeigte, sondern auch in größerer Menge strahliert werden konnte.

a) Mit der unter 2a beschriebenen Methode wurden die Viskositäten aus der Durchlaufzeit bestimmt. Es ergaben sich die in Tabelle II angegebenen Werte, bei denen die für die Berechnung notwendige Konstante aus den für Wasser bei 20°C gemessenen Durchlaufzeiten mit Hilfe der bekannten Viskosität  $\eta_{H_2O} = 0,01002$  cP bestimmt wurde (mittlerer rel. Fehler etwa 1%).

Die für das Blutserum des Hausschweines gemessenen Werte liegen innerhalb des für Humanum von Rauch (1964) angegebenen Viskositätsbereiches.

b) Die nach Methode 2b gemessene Verschiebungsgeschwindigkeit des Flüssigfadens ist in Abb 2a als Funktion der Druckdifferenz  $\Delta p$  dargestellt. Sowohl für die Chromschwefelsäure-Aszessmischung (5/7, gestrichelt) als auch für H<sub>2</sub>O (durchgezogen) ergeben sich Ursprungsgerade. Für Endo- und Perilymphe dagegen ist dies nicht der Fall. In Abb 2b sind Meßwerte dafür im Vergleich mit H<sub>2</sub>O und 5/7 an-

gegeben. Bei kleinen Drucken bis 5 cm Ws bewegt sich der Flüssigkeitsfaden aus Lymphe gar nicht. Bei Drucken bis 10 cm Ws bleibt der Faden häufig hängen. Erst oberhalb  $\Delta p = 10$  cm Ws kann einigermaßen sicher gemessen werden. Die Geschwindigkeit  $v$  steigt dann mit wachsen dem Druck etwa so an, wie für H<sub>2</sub>O. Die Neigung der Geraden ist für Lymphe nur wenig geringer als für H<sub>2</sub>O. Endolympe ( $\Delta$ ) und Perilymphe (+) verhalten sich innerhalb der Meßgenauigkeit gleich. Dies wurde auch bei anderen Kapillardurchmessern bestätigt, so daß die Viskositäten von Endo- und Perilymphe als gleich angesehen werden können.

In Abb 3 sind für  $\phi_K = 0,35$  mm (obere 3 Diagramme) bzw. für  $\phi_K = 0,53$  mm (untere 3 Diagramme) weitere solche Abhängigkeiten für Flüssigkeitsfadenlängen  $l_F = 50$  mm (links),  $l_F = 30$  mm (Mitte) und  $l_F = 10$  mm (rechts) dargestellt. Neben Perilymphe, H<sub>2</sub>O und 5/7 wurde auch Blutserum ausgemessen. Perilymphe und Blutserum verhalten sich auch unter den genannten Bedingungen ähnlich wie in Abb 2b. Ist ein Grenzdruck  $\Delta p_G$  (Schnittpunkt der punktiert eingetragenen Asymptote mit der  $\Delta p$ -Achse) überschritten, zeigt die Abhängigkeit (punktierte Gerade) für die Perilymphe bei allen Meßbedingungen eine etwas geringere Neigung als diejenige für Wasser. Für Blutserum verlaufen die Kurven (strichpunktiert) noch flacher. Dies bedeutet, daß Perilymphe eine etwas größere Viskosität als Wasser besitzt und Blutserum noch eine größere, wenn von dem Effekt des Grenzdruckes zunächst einmal abgesehen wird.

Die nach der Methode 2b bestimmten Visko-

Chromschwefelsäure ist eine Mischung aus H<sub>2</sub>SO<sub>4</sub> (konzentriert) und 2% K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> (wasserfrei).

Die nach der Methode 2b bestimmten Visko-

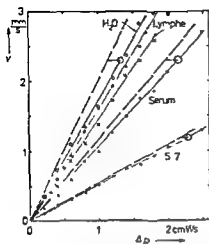


Abb 5 Die am „Miniviskosimeter“ gemessene Verschiebungsgeschwindigkeit  $v$  als Funktion der Druckdifferenz  $\Delta p$  für die Flüssigkeiten 57, Blutserum, Lympe ungefiltert ( $\times$ ) Lympe gefiltert ( $\cdot$ ) und  $H_2O$  (O) Für 57, Serum und  $H_2O$  sind die korrigierten gegen den Uhrzeigersinn verdrehten Mittelwertsursprungsgeraden gestrichelt eingetragen

sitätswerte relativ zu Wasser bei  $39^\circ C$  sind in Tabelle II eingetragen. Sie sind Mittelwerte aus allen nach dieser Methode gewonnenen Ergebnissen, wobei für Lympe und Blutserum die Steigungen der Asymptoten (Abb 3 punktiert bzw. strichpunktiert) der Bestimmung zugrunde gelegt wurden.

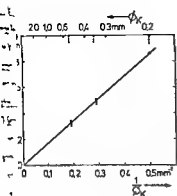
c) Das „Miniviskosimeter“ besitzt nicht nur innen bei der Messung von Flüssigkeit erfüllten I der Kapillare, sondern links und rechts von 1 Zylindern (Abb 1b) noch zwei weitere, von oft durchströmte Kapillarenteile. Letztere besitzen eine Länge von insgesamt 70 mm gegenüber 12 mm von Flüssigkeit erfüllter Kapillarlänge, so daß auch hier, wie unter 2b beschrieben, eine Korrektur durchgeführt werden muß. In Tabelle I sind Werte für den Korrekturfaktor  $k$  angegeben. Abb 5 zeigt die gemessenen Verschiebungsgeschwindigkeiten  $v$  in den Zylindern als Funktion des Druckes  $\Delta p$ . In der Kapillare strömt die Flüssigkeit etwa 60mal schneller. Die durchgezogenen Geraden stellen Ursprungsgeraden dar, welche die Meßpunkte am besten annähern. Bei der Gewinnung von 20 mm<sup>3</sup> Lympe wurde auf eine Trennung von Endolympe und Perilymphe verzichtet. Dies schien erlaubt, nachdem sich bei Messungen mit einem

Gemisch der Lymphen mit Methode 2) Unterschiede gegenüber reiner Peri- bzw. lympe ergeben hatte. Die Messung an filterter Lympe konnte innerhalb von 2 den, die mit gefilterter erst innerhalb Stunden post mortem abgeschlossen v. Für die Flüssigkeiten  $H_2O$ , Serum und 57 die korrigierten Ursprungsgeraden, aus die Viskosität bestimmt wurde, gestrichgetragen. Die relativen Viskositäten, die Art bestimmt wurden, sind in Tabelle I geben.

## 5 DISKUSSION

Endolympe und Perilymphe zeigen gleichhalten bei Kapillarviskositätsmessungen. schiede gehen in der Meßgenauigkeit u-fällig ist das Auftreten eines Grenzdruck bei dieser Methode für Lympe und unterhalb dessen sich der Flüssigkeitsad bzw. stark stockend bewegt. Demnach s: kositätsbestimmungen nach dieser Me-einer einzigen Messung bei einem best-Druck  $\Delta p$  nicht möglich. Eine Ursprun durch diesen Punkt wurde zu falschen u zu großen Viskositätswerten führen. W gegen der Druck über den Grenzdruck  $p_g$  und die Asymptotenneigung als Grund-Viskositätsbestimmung herangezogen d geben sich Werte, die in guter Übereins mit solchen Werten liegen, die mit Methoden gewonnen wurden.

Das Auftreten eines Grenzdruckes ist d-fälligste Abweichung der Druckdifferen-schwindigkeitskurven von Lympe und Ser-genüber denjenigen der Eichflüssigke-Größe hängt nicht von der Flüssigkeits-länge ab (Abb 3), wohl aber vom K-durchmesser (Abb 2b und Abb 3). W-Viskosität von Lympe und Serum e-jenigen von Bingham'schen Flüssigke-gleichbar, so müßte der Grenzdruck  $p_g$  Fadenlänge anwachsen. Daß er dies n-weist darauf hin, daß nur die beiden Faden nicht aber der Faden selbst mit der E-des Grenzdruckes in Zusammenhang F werden können. An den Fadenenden k



6 Grenzdruck  $\Delta p_G$  (aus Fig 2b und Fig 3 entnommen) als Funktion der Kehrwerte des Kapillardurchmessers  $d_K$ . Die Größe von  $d_K$  kann der oberen Skale entnommen werden

dünne Häutchen an der Oberfläche bilden. Sie hängen an der Wand fest und müssen bei Verschiebung des Flüssigkeitsfadens abgerissen und durchbrochen werden. Sie bilden sich aber wieder, sobald die Oberfläche zur Ruhe kommt, und der Flüssigkeitsfaden still steht. Dies ist zunächst eine Annahme. Aus ihr kann jedoch die Abhängigkeit des Grenzdruckes vom Kapillardurchmesser vorhergesagt werden, wenn angenommen wird, daß die Dicke der sich bildenden Häutchen vom Kapillardurchmesser unabhängig ist. Das Fadenende hat näherungsweise die Form einer Halbkugel. Für Hohlräume, bei denen die Wandstärke, d. h. die Hautchendicke  $h$  sehr klein ist gegenüber dem Radius  $r$  der Kugel, ist der Überdruck

$$\Delta p = \frac{2h}{r} \delta_t \quad (8)$$

bei  $\delta_t$  die auftretende tangentielle Spannung. Die Häutchen ist. Wird ihr Grenzwert  $\delta_{tG}$  (beim Reißen) ebenso wie ihre Dicke  $h$  als konstant angenommen, so wird

$$\frac{4h}{d_K} \delta_{tG} \sim \frac{1}{d_K} \quad (9)$$

bei  $d_K$  als Durchmesser der Halbkugel, der Durchmesser der Kapillare eingesetzt ist. Der Grenzdruck mußte also mit wachsendem Kapillar-

durchmesser abnehmen. In Abb 6 ist der den Abb 2b und 3 entnommene Grenzdruck als Funktion des Kehrwertes des Kapillardurchmessers aufgetragen. Die Meßwerte liegen erstaunlich genau auf einer Ursprungsgeraden, wie sie Gleichung (9) vorschreibt, so daß die Annahme der sich bildenden Häutchen als bestätigt angesehen werden kann. Bei großen Oberflächen, d. h. großen Durchmessern wird der Grenzdruck sehr klein, so daß er beim „Miniviskosimeter“ praktisch in der Meßgenauigkeit untergeht.

Die Vorstellung über das Entstehen des Grenzdruckes konnte dadurch bekräftigt werden, daß es gelang, Flüssigkeitsfaden aus Blutserum auch bei Drucken weit unter dem Grenzdruck zu verschieben, wenn beiden Enden je ein kurzer Flüssigkeitsfaden aus Öl angelagert wurde. Allerdings beginnt sich schon nach wenigen Verschiebungen eine Emulsion zu bilden, was sofort zum ursprünglichen Verhalten führt, so daß keine systematischen Messungen durchgeführt werden konnten. Die Lympheflüssigkeit selbst verhält sich demnach wie eine ideale Flüssigkeit, mindestens im untersuchten Scheergefällebereich. Nach Klärung der insbesondere bei Kapillarkviskosimetern auftretenden, stark störenden Nebeneffekte kann zusammenfassend folgendes festgestellt werden.

In Übereinstimmung mit den Angaben von Schneider & Schindler (1964) bzw. von Rauch (1964) werden Viskositäten für Endo- und Perilymphe gefunden, die nur etwa 10% über der von Wasser liegen, jedoch deutlich (Faktor 1,6) unter derjenigen von Blutserum.

Fraulein Dipl. Biologin A. Frei hat bei den Untersuchungen mit dem „Miniviskosimeter“, Herrn Dipl. Ing. G. Appel bei den Untersuchungen mit der Kapillarmethode tatkräftig mitgeholfen. Beiden möchte ich sehr herzlich danken, ebenso Herrn Veterinärdirektor Dr. Schneidewind und seinen Mitarbeitern von der Sanitätsanstalt des Münchner Schlachthofs für ihr verständnisvolles Entgegenkommen. Herrn Dipl. Ing. H. Helle und Herrn Dr. Ing. E. Terhardt bin ich für wertvolle Diskussionen dankbar.

Die Arbeit entstand im Sonderforschungsbereich „Kybernetik“ München, der von der Deutschen Forschungsgemeinschaft unterstützt wird.

## SUMMARY

The viscosity of lymph is determined by using different methods which are discussed especially in regard to the necessary correction coefficient. When using capillaries to measure viscosity, only such data can be taken into account as are produced by a pressure larger than critical. The viscosity of endolymph and of perilymph is found to be equal and 10% larger than the viscosity of water, respectively, yet a factor of 1.6 smaller than the viscosity of serum.

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## PERSTIMULATORY SUPRATHRESHOLD ADAPTATION

### III Sensorineural Deafness

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(Received December 12, 1973)

*Abstract* Perstimulatory suprathreshold adaptation was studied in 243 patients with various types of sensorineural hearing loss. The technique was based on the continuous aural loudness balance—the comparison tone consisting of pulses of 200 msec and intervals of the same length. Measurements were carried out at 20, 40 or 60 dB SL frequencies 1 000–4 000 Hz. In primary hair cell lesions adaptation was slighter than in normally hearing ears and it tended to decrease with increasing threshold. Very pronounced adaptation values were found in cochlear deafness. In central nervous lesions they were normal.

One of the first controlled studies of perstimulatory suprathreshold adaptation was made by Gold (1950). His series included 25 unilateral cases of Meniere's disease. A continuous tone of 40 dB SL (sensation level) was presented at 100 Hz to both ears and the patient instructed to balance the tone in the normal ear so as to match in loudness the one in the diseased ear. A balance tracing showed in 20 cases a decrease of 10 to 50 dB in 3 minutes, indicating increased adaptation in the impaired ear. Palva (1955) used continuous tone balance periods of 15 sec and stimulation of the impaired ear with similar tones for 3 min, and observed large adaptation (30 dB or more) in 14% of 51 purely perceptively deafened ears. In the majority of cases adaptation was slight. These measurements were carried out at 60 and 80 dB above normal threshold for frequencies 500 to 1 000 Hz.

In Pestalozza's modification the loudness level loss was determined with a balance made during 5–10 sec after stimulation of 3 min at 50 dB SL (Cioce & Pestalozza, 1960; Pestalozza & Cioce, 1962), the series included 57 perceptively impaired ears. In normal ears loudness loss of 15 dB was reported for frequencies 1 000–4 000 Hz. In 18 cases of pure end organ lesions adaptation was generally within normal limits or slightly increased in early stages. In VIII nerve lesions (5 cases) the test gave abnormally large values regardless of the degree of hearing loss. In patients with central nervous lesions loudness level loss was within normal limits and abnormal adaptation was reported in only two cases with possible lesions of subcortical structures.

Other reports include those of Pestalozza (1953) and Bosatra (1957), who presented examples of Meniere's disease and toxic end organ lesions associated with large adaptation values. In presbycusis Manzini et al (1956), using Pestalozza's technique on 44 patients, reported that half of the cases showed adaptation within normal values (10–15 dB). The remaining cases showed either pathological adaptation, i.e. loudness level loss of 25–30 dB (22.7% of the patients), or only slightly elevated values. In 18 cases of skull injury Cioce & Spelta (1963) found increased adaptation in presumably central lesions whereas in cases with peripheral lesion the results showed normal or slightly increased values.

This work was aided by a grant from the National Research Council for Medical Sciences.

Table I *Distribution of the material into clinical groups*

Diagnosis	No. of cases
Cochlear lesions	
Noise injury	77
Meniere's disease	29
Streptomycin damage	4
Retrocochlear lesions	
Acoustic neuroma	8
Cerebellopontine arachnoid cyst	1
Central lesions	16
Functional hearing loss	2
Mixed group	
Presbycusis	25
Angiosclerotic degeneration	26
Heredodegenerative deafness	14
Skull injury	14
Non defined sensorineural deafness	27
Total	243

## MATERIAL AND METHODS

The material consisted of 243 adult patients with various types of sensorineural hearing loss (Table I).

In all subjects air and bone conduction thresholds were determined by the usual descending-ascending method using Madsen Model OB 60 audiometer calibrated to ISO 1964 readings. Reception thresholds and speech discrimination were measured as described by Palva (1952). Loudness recruitment was studied by the Fowler or Reger method, or in cases with insufficient threshold differences, taking the amplitude size of the threshold tracings as criterion by a self recording audiometer (Palva, 1957). Threshold tone decay for continuous tones was measured by the selfrecording audiometer during 3 min for the same frequencies as the suprathreshold adaptation.

The apparatus and the testing procedure for measuring suprathreshold adaptation were identical with those described earlier (Kärjä, 1968 and 1970). The measurements were carried out at 40 or 60 dB SL—in a few cases at 20 dB level—at suitable frequencies between 1 000 and 4 000 Hz so that the loudness level did not exceed 95 dB. Determination of air conduction

thresholds with interrupted signal by Stadler Audiometer Model E 800 for frequency studied was followed by prestimulating (Fig. 1). An interrupted pulse stimulus was presented to the test ear at sensation level while a similar comparison stimulus with pulses and intervals of 200 msec

was introduced to the experimental ear for 3 min. Balancing interrupted tone was continued through stimulation. Recovery of the threshold in the test ear was recorded for 60 sec after cessation of stimulation. Adaptation in dB was determined from the difference between adaptation level and the prestimulatory balance level.

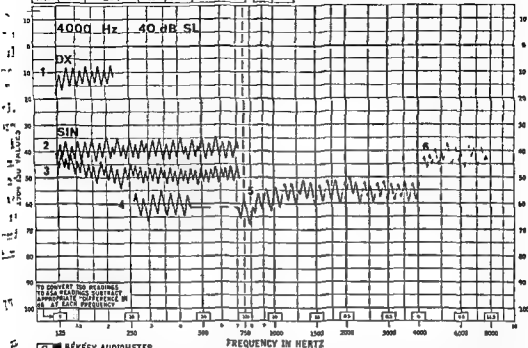
## RESULTS

### Cochlear lesions

This group included 77 patients with noise injury, 29 with Meniere's disease and streptomycin damage. Ages varied from 15 to 57 years and averaged 33.2 years. All had complete loudness recruitment. Speech discrimination rate was in most cases between 70 and 90%, while 4 patients with Meniere's disease and 3 with severe noise injury had scores lower than 50%. Threshold tone decay in 3 min was less than 30 dB in 14 of 77 ears with noise injury and in 10 of 29 ears with Meniere's disease, in which it was between 30 and 47 dB. The results for persistent suprathreshold adaptation in cochlear lesions are shown in Table II. The values were significantly ( $p < 0.05$ ) lower than those measured in the normal material (Kärjä, 1968) and in conductive deafness (Kärjä, 1970). The individual values in cases of streptomycin damage and noise injury are seen in Fig. 2. There was a tendency for adaptation values to decrease with increasing threshold loss. This was not so in patients with Meniere's disease (Fig. 3). The individual adaptation values in all categories showed great variability. Slowly developing adaptation

TRACE	TONE	MASKING	20 dB	dB/SEC	OCTAVE/Min	Test
						YES
COLOR	C.P.	L & R	40	0	1 1/2 2 3 5 10 20	

NAME L. L. NO         
 SEX        AGE 37  
 DATE 2.2.71 TIME        BY J.K.



BÉKÉSY AUDIOMETER  
 GRASON STADLER COMPANY, INC.  
 MODEL NO.        SERIAL NO.       

Perstimulatory suprathreshold adaptation measurement at 4000 Hz 40 dB SL on the left ear of a patient with noise injury. Top left is shown the threshold tracing for the right ear (1). The tracings for the left ear are recorded both with interrupted (2) and continuous test (3) for 3 min. Bottom left is seen the prestimulatory

balancing (4). In the middle the adaptation curve (5) shows loudness level loss of 4 dB in 3 min. The recovery of the post-stimulatory threshold for the left (test) ear recorded with the interrupted test tone is shown on the right (6).

**Table II Cochlear deafness** Perstimulatory suprathreshold adaptation (dB) in 3 min prestimulatory noise level (dB) and post-stimulatory threshold shift (dB) 1 min after stimulation. Stimulus intensity and 60 dB above threshold level

	Stimulus variables											
	1000				2000				4000			
	40		60		40		60		40		60	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
ear deafness												
tation	11.0	7.3	12.8	11.3	15.8	10.0	15.1	13.5	12.1	10.8	8.1	8.4
stimulatory balance	48.2	6.7	62.7	9.8	52.7	8.0	63.5	14.2	42.5	14.1	64.9	9.9
stimulatory												
threshold shift	6.6	4.8	5.9	5.0	3.8	2.2	6.3	4.3	5.6	4.7	5.1	3.6
al ears (Kärjā,												
tation	18.2	13.3	22.6	16.2	21.7	12.3	28.3	17.9	20.5	11.2	28.4	15.0
tation	41.0	8.3	58.9	10.0	40.6	8.4	57.4	11.4	37.9	8.1	57.1	8.2
stimulatory												
threshold shift	3.2	2.7	4.0	2.6	3.6	3.4	4.0	3.2	3.6	3.2	3.3	2.4



Table 1 *Distribution of the material into clinical groups*

Diagnosis	No of cases
Cochlear lesions	
Noise injury	77
Menière's disease	29
Streptomycin damage	4
Retrocochlear lesions	
Acoustic neuroma	8
Cerebellopontine arachnoid cyst	1
Central lesions	16
Functional hearing loss	2
Mixed group	
Presbycusis	25
Angiosclerotic degeneration	26
Hereditodegenerative deafness	14
Skull injury	14
Non defined sensorineural deafness	27
Total	243

## MATERIAL AND METHODS

The material consisted of 243 adult patients with various types of sensorineural hearing loss (Table 1).

In all subjects air and bone conduction thresholds were determined by the usual descending-ascending method using Madsen Model OB 60 audiometer calibrated to ISO 1964 readings. Reception thresholds and speech discrimination were measured as described by Palva.

2) Loudness recruitment was studied by the Fowler or Reger method, or in cases with insufficient threshold differences, taking the amplitude size of the threshold tracings as criterion by a self recording audiometer (Palva, 1957). Threshold tone decay for continuous tones was measured by the selfrecording audiometer during 3 min for the same frequencies as the supra threshold adaptation.

The apparatus and the testing procedure for measuring suprathreshold adaptation were identical with those described earlier (Kärja, 1968 and 1970). The measurements were carried out at 40 or 60 dB SL—in a few cases at 20 dB level—at suitable frequencies between 1 000 and 4 000 Hz so that the loudness level did not exceed 95 dB. Determination of air conduction

thresholds with interrupted signal by Stadler Audiometer Model E 800 for frequency studied was followed by prestimulating (Fig 1). An interrupted pulse stimulus was presented to the test ear at sensation level while a similar comparison stimulus with pulses and intervals of 200 msec was fed to the contralateral ear. The pulse was simultaneous in both ears. When the subject recorded the balance for 30 sec an interrupted, stimulus was introduced into the experimental ear for 3 min. Balancing interrupted tone was continued through stimulation. Recovery of the threshold test ear was recorded for 60 sec of stimulation. Adaptation in dB was calculated from the difference between adaptation and the prestimulatory balance level.

## RESULTS

### *Cochlear lesions*

This group included 77 patients with noise injury, 29 with Menière's disease and streptomycin damage. Ages varied from 57 years and averaged 33.2 years. All complete loudness recruitment phenomenon. Speech discrimination rate was in most cases between 70 and 90%, while 4 patients with Menière's disease and 3 with severe noise injury had scores lower than 50%. Threshold decay in 3 min was less than 30 dB in four ears with noise injury and in two ears with Menière's disease, in which it was between 30 and 47 dB. The results for prestimulating suprathreshold adaptation in cochlear lesions are shown in Table II. The values were ( $p < 0.05$ ) than those measured in the control material (Kärja, 1968) and in conductive hearing loss (Kärja, 1970). The individual cases of streptomycin damage and noise injury are seen in Fig 2. There was a tendency for adaptation values to decrease with increasing threshold loss. This was not so in patients with Menière's disease (Fig 3). The individual variability in all categories showed slowly developing adaptation.

e III Cochlear deafness Amplitudes (dB) of pre- and post stimulatory thresholds, and excursion is of prestimulatory balance and adaptation tracings

	Stimulus variables											
	1 000				2 000				4 000			
	40		60		40		60		40		60	
	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D
<i>ear deafness</i>												
lim threshold	82	29	75	25	69	14	71	20	68	20	67	30
m balance	98	28	91	30	73	26	86	44	74	26	91	32
tation tracing	124	50	98	35	88	38	99	48	88	36	77	26
stim threshold	86	32	72	21	74	27	70	26	73	27	71	31
<i>at ears (Karja,</i>												
<i>-)</i>												
m threshold	60	18	56	19	56	18	52	17	56	17	50	12
m balance	71	29	65	22	73	28	66	23	70	25	66	31
tation tracing	70	26	71	26	71	32	70	26	68	24	68	28
stim threshold	58	18	58	20	68	18	54	20	54	16	54	19

others suffered from localized vascular diseases  
 threshold tone decay (2 000-4 000 Hz) was  
 than 30 dB in all the ears studied. Loudness  
 was incomplete in 7 patients and negative  
 in the others. The values for perstimulatory  
 athreshold adaptation (Fig. 5) were equal

to normal as were also the prestimulatory  
 balance levels and post-stimulatory threshold  
 shifts and the excursion widths of the tracings.

In addition, 2 patients with functional hearing  
 loss were studied. The measurement of supra-  
 threshold adaptation was successful in both even  
 though there was some uncertainty as regards

e IV Mixed group of sensorineural deafness Perstimulatory suprathreshold adaptation, prestimu-  
 balance and post stimulatory threshold shift

explanations see Table II

		Stimulus variables											
		1 000				2 000				4 000			
		40		60		40		60		40		60	
		Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D
with complete recruitment													
a	tation	14.6	5.7	7.3	7.8	14.6	10.4	10.9	12.4	8.4	9.0	13.4	11.9
	mulatory balance	52.3	6.9	66.6	9.4	56.6	16.8	62.4	10.5	52.4	12.4	59.8	9.4
	tumulatory hold shift	5.2	4.4	4.9	4.7	3.9	2.8	5.2	3.2	6.6	5.2	5.8	6.0
with incomplete recruitment													
b	tation	15.7	13.8	23.1	14.2	27.6	7.7	24.6	15.3	40.3	7.6	22.9	15.0
	mulatory balance	56.0	11.0	59.1	8.3	59.0	12.9	61.8	8.9	52.4	11.6	61.1	9.0
	tumulatory hold shift	6.0	5.6	4.6	4.3	7.8	9.0	6.1	5.6	7.2	4.5	6.1	5.1

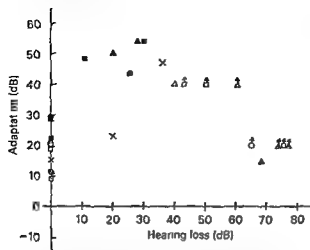


Fig 4 Perstimulatory suprathreshold adaptation on 9 patients with retrocochlear deafness. Arrows indicate the loss of the stimulus sensation  $+ = 40$  dB,  $\times = 60$  dB SL for 500 Hz the squares and the triangles with points 20 dB SL at 1 000 and 2 000 Hz. Other symbols as in Fig 2

determination of hearing thresholds. Adaptation at 40 or 60 dB SL for 1 000 and 2 000 Hz varied between 18–27 dB, being close to the normal means (Table I).

#### Mixed group

This group totalled 106 patients (Table I).

The material was divided into two parts, 56 cases with complete recruitment and 50 cases

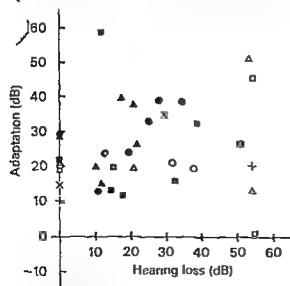


Fig 5 Perstimulatory suprathreshold adaptation in 16 cases with central hearing loss. Symbols are the same as in the preceding diagrams

with negative or incomplete recruitment. The groups included patients from all the etiologic groups.

In the first group there were 4 ears with threshold tone decay exceeding 30 dB or 4 000 Hz. Discrimination scores were between 60–90%. In the second group threshold tone decay exceeded 30 dB in 8 patients, discrimination score was lower than in the first group, in others between 70–90%. In the third group with low speech discrimination score, threshold tone decay was measured. Perstimulatory suprathreshold adaptation in 9 recruiting ears (Table IV) was statistically different from that measured in cochlear lesions ( $P < 0.05$ ) than recorded in the normal ears and in the patients with negative or incomplete recruitment excluding the mean of 4 at 1 000 Hz. The latter group did not differ from normally hearing ears. The individual data are presented in Fig 6.

Pronounced threshold tone decay (more than 30 dB) coincided with large adaptation in patients with incomplete recruitment. In patients with complete loudness recruitment the recruitment did not show this correlation. The two groups did not differ statistically as regards prestimulatory balance levels and poststimulatory threshold shifts, although the means were higher than in normal material. Neither the threshold amplitudes (interrupted), nor the excursion widths of prestimulatory and adaptation tracings differ from normal.

#### DISCUSSION

Suprathreshold adaptation in end organs has generally been found to be close to normal even though so high values as were previously reported (Hood, 1950; Palva 1955; Lozza & Cioce, 1962; Cioce & Speltz; Manzini et al, 1956). Because of measurement techniques used by the above investigators, normal values were lower than those reported by the present author (Kärjä, 1968). Adaptation in primary hair cell lesions in this study was found to be less than in the normally hearing

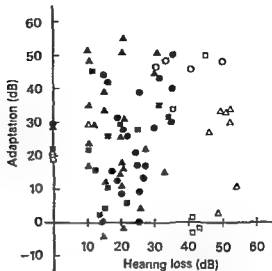
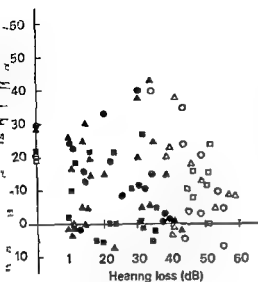


Fig. 5. Perstimulatory suprathreshold adaptation in 56 cases with complete recruitment (left) and in 50 cases

showing negative or incomplete recruitment (right) in the mixed group of sensorineural deafness.

conductive deafness (Kärjälä, 1970), and it tended to decrease with increasing hearing loss. However, adaptation values comparable to normal were found in some ears with slight hearing loss (Fig. 2). This must signify that remaining functioning hair cells in the ears with cochlear damage adapt less in the case of a shift of equal sensation level and of the same sound pressure level than do the corresponding stimulated hair cells in normally hearing ears. In Meniere's disease, regardless of the degree of hearing loss, there were several cases with high adaptation values (Fig. 3). This accords with the results which showed that in most unilateral cases adaptation in the diseased ear was greater than in the healthy comparison ear. This exceptional behaviour in Meniere's disease compared with other inner ear lesions might be due to the fact that the hearing loss is caused by a probable biochemical disorder, not by primary hair cell damage.

In the recruiting ears in a mixed group consisting of various types of sensorineural hearing loss, adaptation was similar to the ears with primary hair cell lesions, lower adaptation values as compared with the normal material, whereas adaptation in ears with incomplete or negative recruitment

indicating a possible retrocochlear lesion tended to be significantly higher. The values in the latter group were statistically higher than those for the ears with complete recruitment. Suprathreshold adaptation in cases with VIII nerve lesions was abnormally high almost without any exceptions. Pestalozza & Cioce have reported comparable results. It seems that in these cases the number of the functioning neurons is too slight to transfer continuous acoustic information, the stimulation is accompanied by a pathologic loudness level loss. Massive lesion of afferent neurons of this kind is seldom possible in higher levels, and adaptation in central nervous lesions is thus normal.

In verified VIII nerve lesions, and in ears with negative recruitment suggesting retrocochlear deafness, perstimulatory suprathreshold adaptation of high degree was associated with pronounced adaptation at threshold level. In other etiologic categories no correlation was found between the two tests. A considerable loudness level loss could be associated with only a slight adaptation at threshold and cases with slight adaptation or none at suprathreshold level could show any amount of tone decay at threshold.

Prestimulatory balance levels of recruiting

ears tended to be higher than in normally hearing material. This is apparently due to the recruitment phenomenon, for equal loudness in the control ear the intensity of the comparison tone must be greater than the stimulus intensity in the recruiting test ear.

The excursion widths of threshold amplitudes and of prestimulatory balance and adaptation tracings tended to be wider than in the normal material. The normally hearing subjects participated in a greater number of test runs and were thus more familiar with the recording technique. As is well known, the excursion amplitudes of the threshold tracings registered with continuous test tones are in most cases less than 5 dB for recruiting ears at frequencies 2 000 Hz or more. This is not the case with interrupted test tone as used in the present study. The reasons may be that the threshold level for a continuous test tone caused by adaptation is poorer than for an interrupted tone and the recruitment phenomenon is more pronounced at higher intensities than close to the threshold minimum.

## ZUSAMMENFASSUNG

Perstimulatorische überschwellige Adaptation wurde an 243 Patienten mit verschiedener Schallempfindungsschwerhörigkeit geprüft. Die Messtechnik gründete sich auf binauralen, dauernden Lautstärkevergleich. Der Reiz bestand aus 200 msec langen Impulsen und langen Intervallen. Stimulusintensität war 20, oder 60 dB über dem Schwellenwert, für die Frequenz von 1 000–4 000 Hz. Bei Haarzellenbeschädigungen war die Adaptation geringer als in den normalhörenden Ohren. Sehr grosse Adaptationswerte wurden

bei retrocochleären Störungen gemessen. Bei Schwerhörigkeit war die Adaptation normal.

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## MONITORING THE POST-MASTOIDECTOMY STATUS BY MEANS OF RADIOACTIVE STRONTIUM (Sr-85)

R. Härmä and P. Karjalainen

*From the Department of Otolaryngology, University Central Hospital, Kuopio, Finland*

(Received December 13, 1973)

**act** Stimulation of postoperative calcium metabolism in the mastoid process was studied by means of active strontium (Sr 85) in a series of 52 patients underwent an ear operation. Calcium metabolism reached maximal values within 3 months began to ase after 11 months and reached the normal level e course of 2 years. Patients subjected to ear surgery id therefore be followed up for at least 2 years operatively before the outcome in respect of osseous ges can be regarded as permanent.

use of radioisotopes in the diagnosis of e bone diseases has gained increasing clinical ortance in recent years. Bone-seeking isotopes to locate and determine quantitatively the ulation of bone calcium metabolism induced a local osseous process such as a malignant iour, osteitis or a fracture. There is intensive ake of bone seeking calcium, strontium, fluor, at sites of new bone formation. As almost osseous processes show new formation in ition to resorption, it is usually possible to onstrate and measure the bony processes h radioisotopes of these substances. The ie method may also be employed to w when the osseous process has ended iuma induced local stimulation of calcium abolism can be established with strontium- within the first few days after a trauma onium activity is preserved in cancellous ie for 2 years, though decreasing, it may be ined for 6-9 years in hard bones (Bessler, 197 Hall et al., 1967, Wendeberg, 1961, Vittali Merckling, 1970). Strontium activity behaves

in the same way after bone operations as after bone fractures (Bessler, 1967).

The strontium-85 measurements we made in cases of mastoiditis (Härmä et al., 1971) led to the application of the method to sequelae of middle ear operations. The purpose of this study was to establish with the help of Sr 85 measurement when the bony process after ear surgery has ended in the mastoid process. Objective determination of this time point would give at the same time an excellent indication as to how long operations on the middle ear should be monitored clinically before the outcome can be regarded as permanent.

### MATERIAL AND METHODS

Our material consisted of 52 operated ears. Radical mastoidectomy had been performed on 50 of them and limited atticotomy in 2 cases. The posterior wall of the auditory meatus had been left intact in 23 patients subjected to 50 radical operations. There were 15 cases in which it had been freed temporarily during the operation and been replaced, or the operative cavity had been filled with bone chips chiselled from the cortical layer of the mastoid process. Two or more investigations had been made on six ears, making a total of 64. The earliest cases had been measured a few days, the most recent 55 years postoperatively. The investigation material was so selected that the control ear of

ears tended to be higher than in normally hearing material. This is apparently due to the recruitment phenomenon, for equal loudness in the control ear the intensity of the comparison tone must be greater than the stimulus intensity in the recruiting test ear.

The excursion widths of threshold amplitudes and of prestimulatory balance and adaptation tracings tended to be wider than in the normal material. The normally hearing subjects participated in a greater number of test runs and were thus more familiar with the recording technique. As is well known, the excursion amplitudes of the threshold tracings registered with continuous test tones are in most cases less than 5 dB for recruiting ears at frequencies 2 000 Hz or more. This is not the case with interrupted test tone as used in the present study. The reasons may be that the threshold level for a continuous test tone caused by adaptation is poorer than for an interrupted tone and the recruitment phenomenon is more pronounced at higher intensities than close to the threshold minimum.

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*Abstract* Stimulation of postoperative calcium metabolism in the mastoid process was studied by means of radioactive strontium (Sr 85) in a series of 52 patients who underwent an ear operation. Calcium metabolism reached maximal values within 3 months, began to decrease after 6 months and reached the normal level in the course of 2 years. Patients subjected to ear surgery should therefore be followed up for at least 2 years preoperatively before the outcome in respect of osseous changes can be regarded as permanent.

The use of radioisotopes in the diagnosis of bone diseases has gained increasing clinical importance in recent years. Bone-seeking isotopes enable us to locate and determine quantitatively the stimulation of bone calcium metabolism induced by a local osseous process such as a malignant tumor, osteitis or a fracture. There is intensive uptake of bone seeking calcium, strontium, fluorine, at sites of new bone formation. As almost all osseous processes show new formation in addition to resorption, it is usually possible to demonstrate and measure the bony processes with radioisotopes of these substances. The same method may also be employed to determine when the osseous process has ended. Gamma-induced local stimulation of calcium metabolism can be established with strontium-85 within the first few days after a trauma. Strontium activity is preserved in cancellous bone for 2 years, though decreasing, it may be used for 6-9 years in hard bones (Bessler, 1967; Hall et al., 1967; Wendeberg, 1961; Vittal, 1967; Merckling, 1970). Strontium activity behaves

in the same way after bone operations as after bone fractures (Bessler, 1967).

The strontium 85 measurements we made in cases of mastoiditis (Härmä et al., 1971) led to the application of the method to sequelae of middle ear operations. The purpose of this study was to establish with the help of Sr-85 measurement when the bony process after ear surgery has ended in the mastoid process. Objective determination of this time point would give at the same time an excellent indication as to how long operations on the middle ear should be monitored clinically before the outcome can be regarded as permanent.

### MATERIAL AND METHODS

Our material consisted of 52 operated ears. Radical mastoidectomy had been performed on 50 of them and limited atticotomy in 2 cases. The posterior wall of the auditory meatus had been left intact in 23 patients subjected to 50 radical operations. There were 15 cases in which it had been freed temporarily during the operation and been replaced, or the operative cavity had been filled with bone chips chiselled from the cortical layer of the mastoid process. Two or more investigations had been made on six ears, making a total of 64. The earliest cases had been measured a few days, the most recent 5.5 years postoperatively. The investigation material was so selected that the control ear of



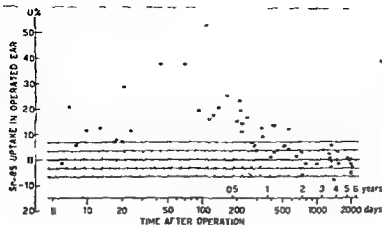


Fig 1 Sr 85 uptake ( $U$ ) in ear operation (o) and in normal ears. mean of normal persons is 0%, 1 hatched areas 1 S.D. and 2 S.D. mean

$$U = \frac{c - c_o}{c_o} \times 100\% \text{ (patients)}$$

$$U = \frac{c_r - c_l}{c_l} \times 100\% \text{ (normal person)}$$

$c$  = counts in operated ear

$c_o$  = counts in healthy ear

$c_r$  = counts in right ear

$c_l$  = counts in left ear

each patient had to be healthy and the postoperative phase of recovery must have been uneventful. Cases in which the ear failed to dry post-operatively were excluded. The control material was comprised of 20 persons free from ear disease.

The measuring method used was a slightly modified version of the one presented earlier (Härmä et al, 1971). The isotope used was Sr-85 as strontium chloride. A dose of 25  $\mu$ Ci was injected intravenously 72 hours before the measurement. The measurement was made with a conventional detector analyser-scaler device. Counts were taken five times on each side separately, with the measuring aperture of the detector at right angles to the mastoid. The measuring distance was changed after each count. The crystal-skin distance was 7 cm and the measuring area on the skin was a circle 5 cm in diameter. The measuring time of one count was 1 minute, making a total of 5 minutes on each side. The room background (patient present) was subtracted from the counts. The result is presented as the difference in counts between the operated and unoperated ears as a percentage of the counts from the unoperated ear. In the control material the difference was measured from the counts of the left and right ears (see caption to the figure). This means that in both materials if the uptake in the latter ear is greater than that in the former, the percentage is negative.

## RESULTS

The results are shown in Fig. 1. Activity rose very sharply in the first 6 months. The highest values were seen in cases in which either the posterior wall of the auditory meatus had been freed temporarily or bone chips had been used to fill the cavity. Activity began to decrease in the first 6 months after the operation and returned to the normal distribution in the course of 2 years. If over 2 years had elapsed from surgery, the measuring results kept within the range of the normal distribution. Activity remained high in the cases in which the ear failed to dry post-operatively, but these cases were excluded from the present series.

## CONCLUSIONS AND DISCUSSION

The stabilisation of calcium metabolism to take place during 2 years concurs with clinical experience and follow up studies. A high incidence of changes in hearing 6 months after ear surgery, even years later, has been reported. Palva et al. stated in 1957 that half a year after obliteration according to the method the ear canals were larger than pre-operatively, and this trend continued to the 2-year point. Two years postoperatively the ear size was the same as at 12 months. The ear

radiograms that new bone was still forming : second and third postoperative year It unlikely that a significant degree of new formation continued in our series over 2 after the operation

: studies published to date on the late of ear operations have been typical / up investigations There has been a lack anty and some disagreement about the in which the postoperative result must be ded as permanent The method described permits objective detection of the cessation e osseous processes in the operative area y be claimed on the strength of our own al that the follow up period for ear opera- must be at least 2 years before the outcome construction of the auditory meatus can be ded as permanent The same is obviously of tympanoplasties, for the position of the y ossicles and the tension of the tympa- s membrane can probably be regarded as lised only when the calcium activity of the lle ear has subsided As we showed in our er study, increased uptake in the unoperated uggests inflammation in the mastoid process thus possible to show with the method also presence of the bony process in the ear ated on 2 years earlier High activity which sts for over 6 months indicates, similarly, the inflammatory process in the operated oid process has not subsided

## ZUSAMMENFASSUNG

Die Stimulation des postoperativen Calciumstoffwechsels im mastoiden Fortsatz wurde mit Hilfe von radioaktivem Strontium (Sr 85) an 52 am Ohr operierten Patienten untersucht Der Calciumstoffwechsel erreicht nach drei Monaten maximale Werte, beginnt nach sechs Monaten zu fallen und zeigt nach zwei Jahren normale Werte Demnach sollten Patienten nach Ohrenoperationen mindestens zwei Jahre lang postoperativ betreut werden, damit das Ergebnis hinsichtlich Knochenveränderungen als permanent gelten kann

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# AN ELECTRON MICROSCOPIC STUDY OF THE TYMpanoJUGULAR GLOMUS

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(Received December 13, 1973)

**Abstract.** Seven temporal bones from four human foetuses of crown-rump length 55-62 mm were prepared for electron microscopy and stepwise sectioned. Seven glomera were found along the tympanic and auricular branches and/or in the adventitia of the superior bulb of the jugular vein. The glomus parenchyma was made up of chief cells characterized by a content of membrane-bound, osmophilic granules 114 nm in mean diameter. The cells were almost completely invested by supporting cells in which nerve fibres were embedded as well. The numerous vessels contained fenestrae in the endothelium. The fine structure of the tympanojugular glomus is similar to that of the carotid body.

Tympanojugular glomus is the term used for the complex of small glomera located along the auricular branch of the vagus nerve and the tympanic branch of the glossopharyngeal nerve in relation to the adventitia of the superior bulb of the jugular vein and in the middle ear.

Osenwasser (1945 and 1968) reported the first recognized case of a carotid glomus tumour-like mass occurring in the middle ear. He suggested that the tumour had developed from the glomus

jugulare described by Guild (1941). So far, knowledge of the morphology of the tympanojugular glomus has been based on microscopy (Guild, 1953; Vara-Thorbeck, Zettergren & Lindström, 1951). Electron microscopy is of interest in order to elucidate whether or not the tympanojugular glomus is morphologically similar to the carotid glomus.

## MATERIAL AND METHOD

Seven temporal bones obtained from four human foetuses designed below by their crown-rump length in millimetres: cr 55, cr 59 and cr 62. Under general anaesthesia the foetuses were removed by hysterotomy. The cord vessel 11-20 ml cold 3% glutaraldehyde buffered with cacodylate to pH 7.4 was used. The area of the tympanojugular glomus was removed. The removed specimens were further fixed for 7-24 hours by immersion into cold

**Fig 1** Light microscopic low power view of a survey section cut parallel to the posterior surface of the petrous portion of the left temporal bone from foetus cr 62. A small glomus (11) is embedded laterally in the superior bulb of the jugular vein (10). The glomus is not in immediate relation to the auricular branch which is situated in the future mastoid canaliculus (12) or to the tympanic branch which is situated on the promontory (9). Basal turn of cochlea (1). Internal carotid artery (2). Superior cervical ganglion (3). Glossopharyngeal nerve area peripheral to the ganglion (4). Inferior petrous sinus (5). Inferior ganglion of vagus nerve (6). Accessory nerve (7). Loose mesenchyme in the future tympanic cavity (8). Tympanic branch of the glossopharyngeal nerve (9). Internal jugular vein (10). Glomus (11). Auricular branch of the vagus nerve (12).  $\times 35$ .

**Fig 2** Light microscopic micrograph showing a tympanojugular glomus from foetus cr 62. The glomus is situated in close relation to the endothelium of the internal jugular vein (5). The majority of cells are to be chief cells (1) a few supporting cells (3). The glomus is very vascular (2) considering its size.  $\times 480$ .

**Fig 3** Electron microscopic low power view of a tympanojugular glomus from foetus cr 59. Of the thin sectioned through the nucleus two may be seen as chief cells (cc) with regular, relatively pale and one as a supporting cell (sc) with an electron-dense nucleus of a dense chromatin structure. The space between the chief cells is of a highly intricate architecture being composed of chief cell parts (ccp), nerve (nf) and thin processes (arrows).  $\times 4000$ .



Table I *Material, processing, and findings of tympanojugular glomus*

	Foetus cr 55 R side	Foetus cr 55 L side	Foetus cr 59 R side	Foetus cr 61 R side	Foetus cr 61 L side	Foetus cr 62 R side	Foetus cr 61 L side
Stepwise sectioned depth, mm	0.9	1.2	0.8	0.4	1.2	2.0	2.3
No. of survey sections	179	94	110	40	171	237	234
Mean distance between sections, µm	4.9	12.8	7.6	10.8	6.8	8.4	9.7
No. of ultrapyramids without glomus	1	2	1	1	6	0	0
No. of ultrapyramids with glomus	1	0	1	0	1	3	3
Stepwise sectioned parts (in fractions) of the auricular branch and	Medial 1/4 in jugular fossa	Medial 1/3 in jugular fossa	None	Medial 1/3 in jugular fossa	Medial 1/2 in jugular fossa	1/1 in jugular fossa  1/1 in mastoid canaliculus 1/2 in facial canal	1/1 in jugular fossa  1/1 in mastoid canaliculus 2/3 in facial canal
Number of glomera found along or close to the branch	1	0	0	0	0	2	2
Stepwise sectioned parts (in fractions) of the tympanic branch and	None	None	1/1 infra tympanic 2/3 intra tympanic	1/1 infra tympanic	None	1/1 infra tympanic 2/3 intra tympanic	1/1 infra tympanic 1/2 intra tympanic
Number of glomera found along the branch	0	0	1	0	0	0	1

uteraldehyde, rinsed in 0.05 M cacodylate buffer, kept for about 24 hours in sucrose, and post fixed for 1 hour in 1% osmium tetroxide. The specimens from foetuses cr 55 and 61 were dehydrated in increasing concentrations of ethyl alcohol and carried through propylene oxide to be embedded in Maraglas (Freeman & Spurlock, 1962), specimens from foetuses cr 59 and cr 62 were dehydrated in acetone and carried through propylene oxide to be embedded in Araldite. The specimens were placed with the posterior surface of the petrous portion of the temporal bone on the flat lid of Beem plastic capsules No. 00 whose pyramid-shaped end, generally used for embedding, had been cut off. The blocks were polymerized for 48 hours at 60°C.

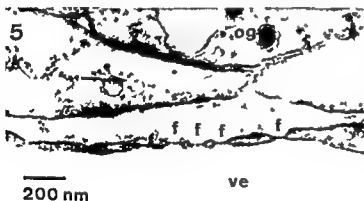
The blocks were trimmed in part manually with cleaned razor blades and in part with a

Reichert TM60 machine, equipped to make survey sections and ultrathin sections. Survey sections and ultrathin sections were cut on a Reichert OMU2 ultramicrotome mounted with glass knives. The 1 µm sections used for light microscopy were stained with basic toluidine blue for 1–2 minutes on a hot plate at about 80°C and mounted on slides. Ultrathin sections were collected on support grids coated with 1.4% Formvar. The sections were double-contrasted with zinc uranyl acetate and lead citrate (Venable & Coggeshall, 1968). The ultrathin sections were studied and photographed in a JEM-T7 electron microscope.

To locate the glomera, specimen blocks stepwise sectioned. Each mounted survey section was assessed in the light microscope for the presence of glomus tissue, and the depth of the nerve was based upon this assessment. If a



*Fig 4* Electron microscopic medium power view showing organelles in a tympanojugular glomus chief cell from foetus cr 67. The chromatin is granular and concentrated predominantly in fragments (*ch*) which are partly scattered in the nuclear ground substance and partly accumulated beneath the nuclear membranes. In the cytoplasm Golgi zone (*g*) whose only cistern contains electron opaque material of an appearance like the content of the osmiophilic granules (*og*), mitochondria (*m*), granular endoplasmic reticulum (*ger*), vacuoles with pale content (*va*), dense body (*db*) and cilium (*ci*)  $\times 14\,000$ .



*Fig 5* Electron microscopic high power view showing endothelium in a tympanojugular glomus vessel from foetus cr 62. A chief cell part with osmiophilic granules (*og*) is separated from the vessel lumen (*ve*) by a non granular cell process (arrow) and the fenestrated endothelium (*f*)  $\times 57\,000$ .

ion contained cells suspected of being glomus. A pyramid for ultramicrotomy was trimmed. Table I summarizes the processing of the specimen blocks. The findings of tympanojugular glomera and characterizes the searched areas of the temporal bones. Measurements on the micrographs were done with a Zeiss particle size analyser TGZ 3.

## RESULTS

Seven tympanojugular glomera were found and studied in the electron microscope. Two were situated in close relation to the tympanic branch, one in its course between the inferior ganglion of the glossopharyngeal nerve and the tympanic canaliculus and one on the promontory in the

middle ear. Two glomera were situated in immediate relation to the auricular branch on its course in the jugular fossa, and three periauricularly in the superior bulb of the jugular vein without contact with the auricular branch (Fig. 1).

In the light microscope (Fig. 2) the organs were about  $60 \times 45 \mu\text{m}$ , and were made up mainly of cells with rounded, pale nuclei, and ample cytoplasm (chief cells), and a few cells with smaller, dark, irregular nuclei and scanty cytoplasm (supporting cells). Thin walled vessels were numerous, except in glomus cr 55.

In the electron microscope (Figs. 3, 4, 5) the glomera were made up of vessels, nerve fibres, and two types of cell, granular chief cells and non granular supporting cells. The nuclei of the supporting cells were irregular, often semilunar, and relatively dark, surrounded by a narrow rim of perinuclear cytoplasm containing a small Golgi zone and a few ribosomes as well as mitochondria. It was characteristic of this type of cell that it gave off thin cytoplasmic extensions embracing chief cells and/or nerve fibres.

The nucleus of the chief was usually rounded and ovoid. The chromatin fragments were irregularly distributed, but the chromatin fragments always accumulated beneath the nuclear membrane and close to nucleoli. The perinuclear cistern was closed in punctate spots by clear pores. The mitochondria were oval or

shaped with transversely placed crests and a few granules in the matrix. The rough endoplasmic reticulum was scanty, occurring partly in the form of solitary, flat vesicles and partly as a few parallel lamellae. Similar small vesicles and lamellae had no ribosomes attached to their outer surface. Non membrane bound ribosomes occurred in major numbers usually collected in small groups. The cytoplasm contained lysosome like bodies of different types and microtubules. Cilia of a  $9 \times 2 + 0$  structure were observed in a very few cells in whose cytoplasm they were deeply invaginated. Where two chief cells adjoined, desmosome-like structures might occur. The striking feature was vesicles which at some distance surrounded an electron dense,

almost homogeneous, central core. These nuclei showed quite some variation in appearance and size. The mean diameter in 837 grains measured was 114 nm, standard deviation.

In all glomera nerve fibres were present. Major bundles were observed even in low power views, and at higher magnification numerous nerve fibres were seen to be wedged in between chief and supporting cells. No synapses were seen.

The exact mutual relation between chief cells, supporting cells, and nerve fibres was difficult to interpret, especially in areas predominated by cell processes. This was due partly to the delicate architecture and partly to the constant presence of cell processes which were difficult to identify. As a main rule, chief cells either joined other chief cells or else were surrounded by thin, non granular cell processes. It was in very limited areas that the plasma membrane of the chief cells was in direct contact with endothelium of the vessels, nerve fibres, or with wide intercellular spaces.

The glomic vessels consisted of a thin layer of endothelial cells, partially surrounded by vascular cells. The endothelium contained a few fenestrae. The vascular lumina were separated from the chief cells by pericytes, as by supporting cells, but a closer contact also occurred.

## DISCUSSION AND CONCLUSION

Based on light microscopy Guild (1953) concluded that the histological structure of tympano-jugular glomus was like that of the carotid body, however, stressed that his method of preparation of the temporal bone material imposed limitations on this conclusion. Compared with the fine structure of the carotid body in human foetuses (Kjærgaard, 1973) the electron microscopic cytological details of tympano-jugular glomus revealed no essential morphological differences. No characteristic section from one of these structures (tympano-jugular glomera) can be distinguished from

tion of the other (i.e. carotid body)" Guild  
 ■ In the era of the electron microscope  
 ■ still valid

# ZUSAMMENFASSUNG

n Schläfenbeine von vier menschlichen Foten  
 itel-Steiss Länge 55–62 mm) wurden für die Elektro-  
 iroskopie präpariert. Es wurden sieben Glomera  
 den, die in der Nachbarschaft des N. tympanicus  
 des II. auricularis und/oder in der Adventitia des  
 is venae jugularis superior lagen. Das Glomus-  
 chym bestand hauptsächlich aus Hauptzellen,  
 kterisiert durch ihren Gehalt an osmiophilen  
 ula mit einem durchschnittlichen Vesikeldurch-  
 r von 114 nm. Die Zellen waren fast vollständig  
 tuttzellen eingeschleitet, die auch Nervenfasern  
 elten. Das Endothel der zahlreichen Gefässe war  
 riert. Die Feinstruktur des Glomus tympanoju-  
 ■ gleicht der des Glomus caroticum.

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## RESPONSES OF RESISTANCE AND CAPACITANCE VESSELS IN FELINE NASAL MUCOSA TO VASOACTIVE AGENTS

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**Abstract** Blood flow from the mucosa of the right nasal cavity was measured with a flowmeter connected to the vein passing through the pterygopalatine foramen in cats.

These two methods could provide information about responses evoked in the resistance vessels (mainly small arteries and arterioles) and in the capacitance vessels (mainly venous vessels and sinusoids). Close arterially administered infusions of angiotensin, noradrenaline, adrenaline and dihydroergotamine elicited constrictions in the resistance as well as the capacitance vessels. Analysis of the ratio of the resistance and the capacitance responses permitted an evaluation of the relative effectiveness of the drugs in constricting the two types of vessel. The constrictor action on the capacitance vessels was in the order Dihydroergotamine > the catecholamines > angiotensin. The order was reversed as for the constrictor action on the resistance vessels.

Measurements of blood flow, arterial and venous pressure, and changes of tissue in various regions, such as skeletal muscle, skin, or intestine, it has been possible to analyse in detail the various peripheral vascular functions, i.e. of the resistance vessels with a precapillary and a postcapillary section, the precapillary sphincter vessels, the capillary exchange vessels, the capacitance vessels and, in some tissues, the shunt vessels (Mellander & Johansson, 1968; Folkow & Neil, 1971). The same vascular functions seem to be present in the nasal mucosa. Previous studies describe effects of autonomic nerve stim-

ulation and drug application on mucous blood flow and patency in the cat, the latter measured by a balloon technique (Malm, 1973a and b). In the present critical analysis was performed to investigate to what extent regional blood flow and recordings combined with this balloon technique might provide direct information about changes in the resistance and capacitance of the nasal mucosa as evoked by certain vasoactive agents.

### METHOD

The results were obtained from experiments on cats, which were anaesthetized with chloralhydrate (about 80 mg/kg i.v.) after induction with thiopentone. Tracheotomy was performed to facilitate spontaneous respiration. Venous blood flow changes in nasal patency were recorded as described previously (Malm 1973) with some recent modifications. In brief, the experimental approach was as follows. The cat was lying on its back with its mouth partly open in a fixed position. To reach the vein which carries the nasal mucosa through the pterygopalatine foramen, the mucosa of the hard palate was reflected free and part of the underlying bone was drilled away. A thin polyethylene catheter (diameter 0.6 mm, length 2 cm) was inserted into the vein near the dorsal surface of the incisor, the right molar tooth and carefully advanced until the tip of the catheter was en-

This investigation was supported by grants from the Swedish Medical Research Council (B74-14X 2210-08C) and C. O. Lundberg's Foundation.

from the pterygopalatine foramen. The vein ligated around the catheter close to the men. It was connected to another catheter of larger bore (inner diameter 1.7 mm, length 10 cm), diverting the venous blood flow through optical blood flow recorder unit, after which it is returned to the animal via a funnel connected to the right jugular vein. Blood flow was continuously recorded on kymograph paper. The tip of the venous outflow catheter was usually at a level 6 to 11 cm above the pterygopalatine foramen, but could be adjusted if desired (see Fig. 1). Changes of nasal patency were estimated from pressure changes in a water-filled catheter placed in the right nasal cavity. The catheter was connected via a polyethylene tubing to an electronic pressure transducer (EMT 33, Elema-Schonander) for recording on a polykymograph (Mingograph, M81, Elema-Schonander).

The pressure bottle and the system was then closed. Arterial blood pressure was recorded from the left femoral artery via a mercury manometer connected to the kymograph. Close arterial infusions were made through the right lingual artery in retrograde direction via a polyethylene catheter. The catheters were heparinized just before the insertion. The cervical sympathetic nerve, and as a rule also the right vagus nerve, were cut.

The following drugs were administered close to the nasal mucosa through the lingual artery as described, via a constant infusion pump: 1) adrenaline bitartrate and 2) noradrenaline bitartrate (0.001, 0.01, 0.1 and 1  $\mu\text{g/kg}$  b.w.), 3) angiotensin-II-amide (Hypertensin N\*, 0.001, 0.01, 0.03 and 0.1  $\mu\text{g/kg}$  b.w.), and 4) dihydroergotamine-methan-sulphate (Orstanorm®, Sandoz), (1 and 10  $\mu\text{g/kg}$  b.w.).

Data are given as mean values  $\pm$  S.E.M. Student's *t*-test was used for statistical evaluation.

## RESULT

### *Evaluation of Techniques for Recording Resistance and Capacitance Functions*

#### *Venous blood flow at rest*

It was essential to define first the region from which blood was drained by the present catheterization technique and this was done by the following procedures. Towards the end of the experiment, or when the cat was dead, infusions of a dye (methylene blue) or of a roentgen contrast medium (barium sulphate) were given in retrograde direction through the venous outflow catheter to study the vascular distribution of these tracer agents either by direct inspection or by angiograms. When the tracers were given under a slight infusion pressure without marked inflow resistance they were distributed almost exclusively to the nasal mucosa of the right cavity, as can be seen in Fig. 1A (barium sulphate). With a somewhat higher infusion pressure which overcame a certain inflow resistance, the solutions were forced over to the mucosa of the left cavity and its major draining vein, as demonstrated in Fig. 1B. The methylene blue method gave similar results. The conclusion is that the right pterygopalatine vein drains blood from the nasal mucosa alone, and normally only from that of the right cavity. It cannot be excluded, however, that a fraction of the mucosal blood flow on the right side is drained by some other small veins, the extent of which may depend on venous outflow pressure.

Blood flow rates were therefore measured at different levels of venous outflow pressure in some cats. It was established that at each given level of venous outflow pressure the blood flow rate was constant throughout prolonged observation periods. It was also found that the blood flow rate was much faster at the low venous outflow pressures than at the high. The blood flow rate was, for instance, about twice as big when the venous outflow catheter tip was set at the level of the nasal cavity (approximately heart level) as when the outflow catheter was 6 to 8 cm above heart level. It must be added that the resistance in the venous outflow catheter was



*Fig 1 (A)* Angiogram of the skull of a cat (submento vertical projection) after barium sulphate injection through a catheter inserted into the vein passing the right pterygopalatine foramen. The opaque medium is distributed almost only to the nasal mucosa of the right cavity



*Fig 1 (B)* Angiogram after barium sulphate administration applied at higher injection pressure than in Fig 1A. The medium is forced into a larger area of the mucosa on the right side and also to the left cavity and to the vein passing the left pterygopalatine foramen

not entirely negligible but caused a of 1 to 3 cm  $H_2O$  at the blood flow countered in the present study

The normal pressure in the veins was estimated to be about 7 cm  $H_2O$ . Flow rates were therefore measured of cats with the outflow catheter 6 to heart level. In these experiments nas was also measured by the balloon technique. Application of a high venous outflow here also served to prevent the s venules and the veins from being by the water pressure (4 cm) in th which is important for the study of capacitance responses (cf Öberg 1967). Under circumstances, the venous blood flow  $0.6 \pm 0.1$  ml/min (6 cats). It is possible that higher flow value at the low venous pressure can be explained by blood from the mucosa not only in the right in the left cavity. The flow value at the outflow pressure of 6 to 8 cm  $H_2O$  therefore more truly reflect flow from the the right cavity alone. Since the wet the mucosa in the right cavity, determined careful dissection was found to a  $\pm 0.04$  g, resting blood flow in the nas can be estimated to be in the range of ml/min  $\times 100$  g tissue

#### *Estimation of changes of mucosal tissue*

A change of tissue caused for instance change of regional blood volume response), is considered to be represented by a pressure change in a balloon inserted in the cavity. This conclusion was based on findings in model experiment tests in situ.

A model of the nasal cavity and of the sinuses that in the cat was designed for the balloon technique. The model consisted of a latex tube surrounded by a glass tube of a somewhat larger diameter. The latex and glass tubes were connected to each other at one end and the interspace (simulating the nasal cavity) was filled with water. The volume of the interspace could be changed by injections of water from a syringe, the plunger of which was 2

a micrometer screw. A water filled balloon, mounted around a polyethylene catheter with sections to the latter designed for cat nose pieces (for details see Malm, 1973a), was positioned inside the latex tube. Balloon pressure in the control period was set to 4 cm H<sub>2</sub>O (as in cat experiments). Pressure variations inside the balloon were followed via the catheter as the pressure in the mentioned interspace (model of nasal mucosa) was altered. A volume change in the latter of 0.016 ml was found to cause a pressure change of 1 cm H<sub>2</sub>O in the balloon and there was a linear relationship between volume and pressure changes within the range of pressure variations observed in the cat experiments ( $\pm 3$  H<sub>2</sub>O, see below).

The balloon technique was also tested in vivo by studying the effects of abrupt changes in regional venous pressure causing passive capacitance responses. The cat was then placed on a table and the vascular bed of the nasal mucosa, the vein through the pterygopalatine plexus being intact, was exposed to given short-term hydrostatic loads by adjustments of the height of the nasal region below heart level. In the experiments balloon pressure increased with increasing hydrostatic load (i.e. regional blood volume) and vice versa.

### *Effects of Drugs*

In the first series of experiments (8 cats) the vascular effects of angiotensin, noradrenaline, and adrenaline were compared when given close arterial to the nasal mucosa as short term graded infusions of 1 min duration. Three to 4 different doses of each drug were given in each experiment within the ranges mentioned in the method section.

At suprathereshold doses, these drugs always evoked a decrease of regional venous blood flow, and occasionally when the larger doses were given an increase of arterial blood pressure. The lowest dose (0.001  $\mu\text{g/kg b.w.}$ ) was often subthreshold with regard to the vascular response to noradrenaline and adrenaline, but usually not to angiotensin. Regional vascular resistance was expressed in the conventional way in peripheral

resistance units (perfusion pressure, mm Hg/regional venous blood flow, ml/min) and the responses of the resistance vessels to the drugs as the per cent change of vascular resistance from the control value before drug administration.

The nasal patency was always increased by suprathereshold doses of the drugs, as evidenced by a fall of pressure in the balloon in the nasal cavity. Such pressure changes, as will be discussed, can be taken as a measure of evoked constrictor responses in the capacitance vessels of the nasal mucosa. They may reflect such effects in at least a semi quantitative way since, as indicated by the model experiments, the pressure changes were linearly related to evoked changes of cavity volume. The elicited capacitance responses can be expressed in terms of pressure changes in the balloon (cm H<sub>2</sub>O).

Fig 2 (A & B) shows the mean resistance and capacitance responses for all infusions of angiotensin, noradrenaline, and adrenaline when given in suprathereshold doses. The amount of angiotensin for all infusions averaged 0.04  $\mu\text{g/kg b.w.}$ , of noradrenaline 0.36  $\mu\text{g/kg b.w.}$  and of adrenaline 0.26  $\mu\text{g/kg b.w.}$  With this compilation of data, the average constrictor responses of the resistance and capacitance vessels were not significantly different for the three agents. To evaluate in greater detail the two vascular responses in relation to each other and to the varying amounts of the drugs, the constrictor effects were expressed as the ratio between the resistance response (per cent increase of resistance above control) and the capacitance response (cm H<sub>2</sub>O pressure fall in the balloon). With such a representation, the relative effectiveness of each drug on the resistance and capacitance vessels can be revealed. The higher the numerical value of the ratio, the greater the relative constrictor action of the drug on the resistance vessels compared to that on the capacitance vessels. The mean value for these ratios are shown in Fig 2C. There was a significant difference between the ratios for angiotensin and noradrenaline ( $p < 0.05$ ) for angiotensin and adrenaline ( $p < 0.01$ ), but not for noradrenaline and adrenaline.

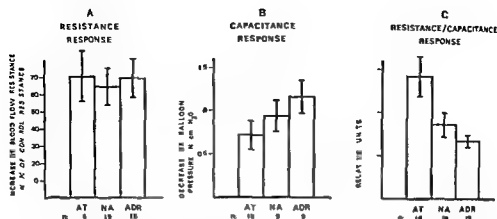


Fig 2 Mean values ( $\pm$ SEM) of the responses in the resistance (A) and the capacitance (B) vessels after infusions of angiotensin (AT) noradrenaline (NA) and

adrenaline (ADR) observed in 8 cats. The resistance/capacitance responses to the three drugs in panel C *n* indicates number of observations.

naline ( $p > 0.2$ ). This implies that angiotensin is a relatively effective constrictor of the resistance vessels, but relatively poor constrictor of the capacitance vessels in the nasal mucosa in comparison with the two catecholamines.

A similar comparative study on the nasal mucosa was performed with noradrenaline and dihydroergotamine (9 cats, 5 of which were the same as in the above-mentioned series). Noradrenaline was administered as described and always given before dihydroergotamine. The latter substance was given once to each cat as an infusion of duration of 1 to 6 min and in total dose varying from 1 to 10  $\mu\text{g/kg b.w.}$  These doses of dihydroergotamine were all threshold

Fig 3 shows such an experiment with noradrenaline infusions and one dihydroergotamine infusion. It can be seen that the responses of the resistance and capacitance vessels to suprathreshold doses of noradrenaline (0.1 and 1  $\mu\text{g/kg b.w.}$ ) were evoked rapidly, and disappeared rapidly after the infusion, whereas the constrictor responses to dihydroergotamine developed more gradual and more prolonged, especially in the capacitance vessels. Note that in equi-resistant doses dihydroergotamine (*d*) evoked a more pronounced capacitance response than noradrenaline (*b*).

Fig 4 (A & B) shows the mean resistance/capacitance responses for all infusions of noradrenaline and dihydroergotamine in the

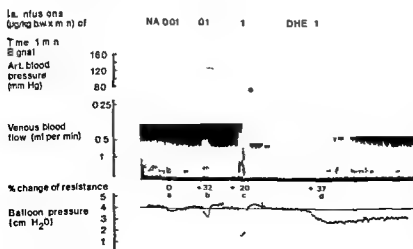
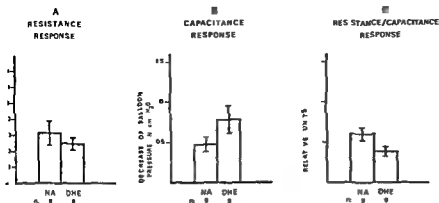


Fig 3 Cat 30 kg. Effects of infusions of noradrenaline (NA) and dihydroergotamine (DHE) on arterial blood pressure, venous blood flow, vascular resistance, and balloon pressure in the nasal mucosa. A decreased balloon pressure is a measure of increased resistance in turn due to constriction of capacitance vessels.



Mean values ( $\pm$  SEM) of the responses in the resistance (A) and the capacitance (B) vessels after infusion of noradrenaline (NA) and dihydroergotamine

(DHE) observed in 11 cats. The ratios of resistance/capacitance responses to the drugs are shown in panel C. *n* indicates number of observations.

periments. The average dose of noradrenaline was  $0.07 \mu\text{g/kg}$  b.w. and of dihydroergotamine  $5.2 \mu\text{g/kg}$  b.w. When the data were compared in this way, no significant difference could be revealed between the two drugs as regards effects on the resistance or capacitance vessels. Yet, the ratios of the resistance and capacitance responses calculated for the various observation experiments were found to be different ( $P < 0.05$ ) for the two drugs (Fig. 4C). This indicates that dihydroergotamine is a relatively more potent constrictor of the capacitance vessels than noradrenaline.

## DISCUSSION

The vascular bed of the nasal mucosa differs in several respects from the vascular design in most other regions. Thus, besides the conventional capillary and postcapillary resistance vessels, capillary sphincters, capillary exchange vessels and capacitance vessels, there are arteriovenous anastomoses (Cauna, 1970) and sinusoids, the latter probably positioned between epithelial capillaries and a deeper venous sinus (Ingelstedt & Rundcrantz, 1963). The sinusoids contain smooth muscles in their walls (Nesrekasi, 1969). Filling of the sinusoids with blood apparently influences nasal patency, since such congestion can be pronounced,

they must, together with the venous vessels, contribute in an important way to the vascular capacitance function of the nasal mucosa.

The resistance function controlling regional blood flow and the capacitance function controlling regional blood volume of course are important determinants of nasal mucosa function. There seems to be no investigation from other laboratories in which blood flow in the nasal mucosa has been studied directly, but there are several studies in which changes of nasal patency have been determined (cf. Malcomson, 1959). In the present study an attempt was made to follow the resistance and capacitance functions simultaneously and quantitatively in the cat nasal mucosa, and to analyse the patterns of vascular response evoked by certain vasoactive agents.

The present cannulation technique of the vein passing through the right pterygopalatine foramen seems to permit measurement of blood flow from the nasal mucosa exclusively, although the absolute figures for resting blood flow can only be considered approximate ( $130 \text{ ml/min} \times 100 \text{ mm}^2$  tissue) due to possible minor drainage by other small veins and to the presence of some venous connections with the contralateral nasal cavity. The fact that venous outflow at rest remained constant throughout prolonged observation periods at a given venous outflow pressure (see above) and the high reproducibility of the resist-

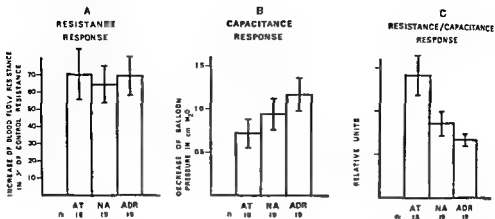


Fig 2 Mean values ( $\pm$  S.E.M.) of the responses in the resistance (A) and the capacitance (B) vessels after infusions of angiotensin (AT), noradrenaline (NA) and

adrenaline (ADR) observed in 8 cats. The resistance/capacitance responses to the three drugs in panel C. *n* indicates number of observations.

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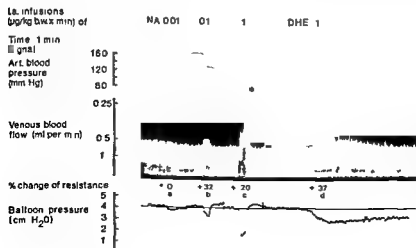


Fig 3 Cat, 30 kg. Effects of infusions of noradrenaline and dihydroergotamine (DHE) on arterial blood pressure, nasal blood flow, vascular resistance, and balloon pressure in the nasal mucosa. A decreased balloon pressure is a measure of increased nasal blood flow in turn due to constriction of capacitance vessels.

sionen von Angiotensin, Noradrenalin, Adrenalin Dihydroergotamin in der Nahe der Nase verursachten Zusammenziehen sowohl in den Resistanz als auch in Kapazitanzblutgefassen. An Hand des Verhaltens der relative Effekt der Substanzen auf das Zusammenziehen beider Arten von Blutgefassen beurteilt man die zusammenziehende Wirkung auf die Kapazitanzblutgefasse ubten in dieser Reihenfolge Dihydroergotamin > die Katekolamine > Angiotensin aus. Fur Resistanzblutgefasse war die Reihenfolge umgekehrt.

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ance responses to a given pharmacological stimulus, indicate that the method permits quite accurate quantitative recordings of changes in mucosal vascular resistance

As evidenced by the model experiment and tests *in vivo*, the present balloon technique seems to provide information, at least in a semi-quantitative way, about nasal patency as affected by responses of the capacitance vessels in the nasal mucosa. This was further corroborated by the fact that the capacitance response to a given pharmacological stimulus was highly reproducible. Changes of nasal patency evoked by physiological and pharmacological stimuli have previously been measured by volumetric recordings of air displacement from or to the nasal cavities after closure of the epipharynx and the nostrils (Tschalussow, 1913, Jackson, 1939). In comparison with the present method, the mentioned technique was found less reliable in that baseline drift frequently occurred, probably due to insufficiency of the cavity closure, the reproducibility of the results was also poorer (Malm, unpublished observations).

Nasal patency can be affected not only by capacitance responses, but also by transcapillary fluid filtration or absorption and by mucosal secretion. Transcapillary fluid movement usually is a relatively slow process (cf Mellander & Ohansson, 1968) and, if present at all in these short term observation periods during drug infusion, such an effect must have been small compared with the observed relatively pronounced capacitance responses. Mucosal secretion was apparently negligible in the present experiments with vasoactive agents, since balloon pressure was found always to return to the control level after cessation of the angiotensin, noradrenaline, and adrenaline infusions. No secretagogue effect of dihydroergotamine has been described. It may be concluded that the acute effects observed with the balloon technique can be ascribed to changes in the capacitance vessels.

The present study showed that angiotensin, noradrenaline, adrenaline and dihydroergotamine all constricted the resistance as well as the capacitance vessels in the nasal mucosa. The analy-

sis of the ratios of the responses of the types of vessel led to the conclusion that the relative effectiveness of the substances in increasing vascular resistance may be listed in the order: Angiotensin > the catecholamines > dihydroergotamine. The relative effectiveness regard to the constrictor response in the resistance vessels, on the other hand, appeared: Dihydroergotamine > the catecholamines > angiotensin. The investigation thus reveals the patterns of vascular response in the series-coupled sections evoked by the vasoactive agents were differentiated and, in the same way as previously described for vascular beds of skeletal muscle and skin (Mellander, 1960, Folkow et al., 1961, Mellander & Nordenfelt, 1970, Jarhult, 1971).

Vasodilatation of the capacitance vessels and a consequent increase of blood content in the nasal mucosa is probably one important factor in decreased nasal patency. The present study therefore may provide a theoretical basis for suggesting that dihydroergotamine, in its preferential and strong constrictor action on the capacitance vessels in doses much smaller than those causing a receptor blockade, may have a beneficial effect in states of nasal obstruction. Clinical research is required to assess a possible therapeutic effect of the drug.

## ACKNOWLEDGEMENT

The author is indebted to Ass. Prof. T. Olsson, Department of Diagnostic Radiology, University Hospital, for making the angiograms.

## ZUSAMMENFASSUNG

Nach regionaler Sympathektomie wurde der Zustand der Mukosa im rechten Nasenraum von einem Strömungsmesser angeschlossen an das Foramen Pterygopalatinum passierende Venen. Ausserdem wurde in den gleichen Nasenraum ein gefüllter Ballon eingeführt, der Druck übertrug. Diese beiden Methoden erlaubten es, gleichzeitigen Veränderungen folgen zu können, somit Aufschluss über das Geschehen in den Blutgefässen (hauptsächlich kleine Arterien und Kapillaren) und in den Kapazitätzblutgefässen (Plexus venosus und Sinusoide) zu erhalten.

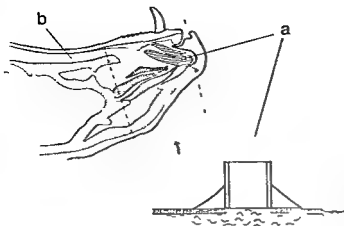


Fig 1 Sagittal section through the head of the cat. Area seen by the detector delineated by dotted lines. Arrow indicates the detector (a) The polyethylene tube fixed over the de-epithelized area on the maxilloturbinal (b) Nasopharynx.

s in nasal patency following Vidian nerve transection. A superior cervical ganglionectomy and in a complete ipsilateral sympathectomy the nasal mucosa of the cat (Ånggård & Den-1974) subsequent stimulation of the Vidian nerve would thus activate parasympathetic fibres selectively.

In the present study, this finding was exploited to study the effects of graded parasympathetic nerve stimulation on the vascular exchange and capacity function in the nasal mucosa were studied simultaneously using a technique described previously (Ånggård & Edwall, 1974). The secretory response in the maxilloturbinal area was studied. The aim of the study was to find if the vascular functions are affected by parasympathetic nerve activation and whether secretory response is accompanied by an increased local blood content. The frequency-response relations for these parameters were studied by graded parasympathetic stimulation. Furthermore, a pharmacological analysis of the local responses was made.

## METHODS

Experiments were conducted on 12 cats weighing 2-3.5 kg and anesthetized with chloralose-urethane (50 mg/kg + 100 mg/kg i.v.). A superior cervical ganglionectomy on one side had been performed 1 or 2 weeks previously, and the Vidian nerve was purely parasympathetic.

The Vidian nerve was exposed by a trans-orbital approach. The eyelids were removed and the eyeball opened and emptied. Periorbital dissection and lateral displacement of the eye exposed the Vidian nerve emerging at the apex of the orbit leading to the sphenopalatine ganglion. The nerve was transected at the apex and freed from surrounding tissues. Minor nerve anastomoses between the ganglion and the trigeminal nerve were cut. Electrical stimulation of the distal end was performed using a bipolar platinum electrode. The nerve and the electrode were covered with Plastibase (Squibb). Monophasic, square wave pulses with a duration of 1 msec and an intensity of 8 V were delivered by a Grass model S 4 stimulator. The trachea was cannulated. The pressure in the femoral artery was measured via a Statham pressure transducer (P 23A) and recorded on a Rikadenki multichannel recorder. Rectal temperature was kept constant at 38°C by means of heating lamps. The posterior auricular artery was cannulated for close intra-arterial infusion into the external carotid artery. The head of the cat was immobilized by means of a steel rod inserted between the jaws and secured in place by dental acrylic.

### *Measurements of tracer disappearance and local blood content*

The technique used to measure changes in the microcirculation was the same as that described in a previous paper (Ånggård & , 1974).

In essence, changes in the exchange and capacitance function were studied by monitoring the local disappearance rate of an easily diffusible tracer,  $^{125}\text{I}^-$  and the changes in the gross pulse rate from iv administered  $^{125}\text{I}^-$  labelled serum albumin

Local disappearance measurements were performed in all experiments  $^{125}\text{I}^-$  as iodide, was obtained as an isotonic carrier-free solution containing  $300 \mu\text{Ci}/\mu\text{l}$  (AB Atomenergi, Nyköping, Sweden)

The epithelium over a small area of the maxilloturbinal mucosa was removed and a small polyethylene tube (PE 90) applied and fixed over the exposed area with an adhesive (Nobecutan, Bofors), thus creating an open well with the subepithelial tissues in the bottom (Fig 1) One hour later, about  $0.3 \mu\text{l}$  of the tracer solution was placed in the well The disappearance of the applied depot was monitored by an external scintillation detector A 5 cm thick lead shield fitting tightly around the head and the nose of the cat had a narrow slit over the right side of the nose and shielded the rest of the cat from the detector (Fig 1) The detector output was fed into two recording channels, each containing a single channel pulse height analyzer and two scalers with digital printout Radioactivity was counted for periods of 40 sec and started immediately after the application of the tracer After each run the final background was determined The net pulse rate (gross pulse rate minus background) was plotted semilogarithmically against time The disappearance rate ( $k$  value), which represents the fractional elimination of the depot per minute, was calculated as follows (Kety, 1949)

$$k = (\log C_1 - \log C_2) / 0.4343 (t_2 - t_1)$$

where  $C_1$  and  $C_2$  are the recorded net pulse rates of the depot at times  $t_1$  and  $t_2$  The time interval  $t_2 - t_1$  is expressed in minutes The calculations for each time interval were carried out using a computer

In 10 cats changes in local blood content of the nose were studied simultaneously with the disappearance measurements  $^{125}\text{I}$ -albumin,

around  $2 \text{ mCi}$  in  $5 \text{ ml}$ , was injected, intravally Gross pulse rate was measured over the nasal cavity as described above Appropriate corrections were made for the overflow of  $^{125}\text{I}$  in the  $^{125}\text{I}$  channel For further details see Änggård & Edwall (1974)

### Measurements of nasal secretion

Nasal secretion was estimated by inspecting the well formed by the polyethylene tube in the disappearance measurements (Fig 1) Using a Zeiss operation microscope it was possible to observe the accumulation of various quantities of secretion When such measurements were performed secretion was measured semiquantitatively - graded as + (+), noticeable +, obvious ++ or rich +

In three experiments the secretion during stimulation was measured quantitatively Changes in local blood content were recorded simultaneously as described above In these studies the epithelium in the bottom of the well was removed The volume required to fill the well was measured, and the secretion during stimulation period was measured in relation to this volume When secretion was rich the volume required to fill the well was measured and was expressed as  $\mu\text{l}/\text{min}$

## RESULTS

### The effects of parasympathetic nerve stimulation on secretion

In all experiments stimulation frequencies of 0.5 or 1.0 imp/sec resulted in a slight increase in secretion Above 2.5 imp/sec secretion was rich, sometimes resulting in an overflow from the nasal cavity When secretion was measured quantitatively (3 cats, 31 stimulations) frequency-dependent secretion was seen from 0.5 to 12 imp/sec, together with simultaneous increases in gross pulse rate The results of a representative experiment are shown in Fig 2

During disappearance measurements, secretion could not be evaluated quantitatively The volume of the applied tracer solution varied in the various applications A rough estimation

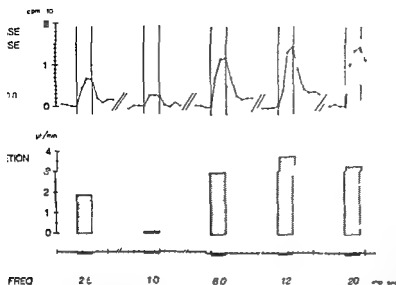


Fig 2 Influence of graded parasympathetic nerve stimulation on the secretory response, and the gross pulse rate of  $^{125}\text{I}$  albumin measured over the right nasal cavity

secretion was possible, however. In all cases where secretion occurred a concomitant increase in the gross pulse rate was seen (Fig 3).

#### *Influence of parasympathetic nerve stimulation on the secretory response and gross pulse rate of $^{125}\text{I}$ albumin*

Stimulation of parasympathetic fibres to the nasal mucosa with frequencies from 0.5 imp/sec resulted in an increase in the  $^{125}\text{I}$ -disappearance rate ( $k$ -value) and gross pulse rate

of  $^{125}\text{I}$  albumin (4 procedures). As secretion interfered with the tracer disappearance measurements, a frequency-response relation could not be investigated. Fig 3 shows a typical experiment where both tracers were studied at the same time as the secretion. A 60% increase in the  $k$ -value was seen at 0.5 imp/sec. Subsequent stimulation with 1.0 imp/sec did not result in a further increase. After an initial increase in the  $k$ -values during stimulation, a decrease was often noted. This decrease was reduced after administration

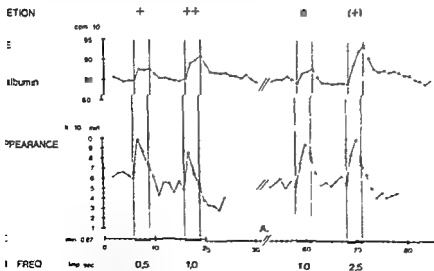


Fig 3 Influence of parasympathetic nerve stimulation on the secretory response and the gross pulse rate of  $^{125}\text{I}$  albumin measured over the right nasal cavity

The secretory response is indicated above the curves. None 0 Slight (+) Noticeable ++ Obvious +++

2.4 kZ. (A) Intra-arterial infusion of atropine.

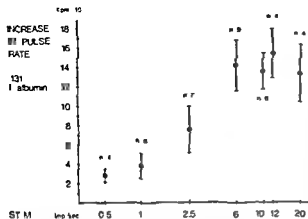


Fig 4 Influence of parasympathetic nerve stimulation on the gross pulse rate of  $^{131}\text{I}$  albumin measured over the right nasal cavity. Results obtained from 10 cats. Mean, standard deviation and number of observations are shown.

of atropine. When secretion occurred on stimulation a reduction of the  $k$  values after the stimulation was observed and at stimulation frequencies above 2.5 imp/sec the secretion made disappearance measurements unreliable. After blocking secretion with atropine these effects were not seen (Figs 3 and 5). Therefore it seems that the reduction in disappearance rate during or after stimulation was due to a dilution of the tracer depot by accumulated secretion. A leakage of the tracer from the well to areas where the mucus had not been removed might also contribute to this effect.

Measurements of the local blood content, as studied by  $^{131}\text{I}$  albumin, showed frequency de-

pendent increases in gross pulse rate continuously with responses in disappearance (Fig 3). In three experiments stimulation frequencies between 5–20 imp/sec were used (stimulations) and frequency dependent increases were seen in gross pulse rate (Figs 2 and 3). At 0.5 imp/sec a clear increase was always seen and major changes in gross pulse rate in the frequency range 0.5–6 imp/sec.

To investigate the maximal effects of parasympathetic and sympathetic stimulation on gross pulse rate, maximal parasympathetic stimulation was compared to the maximal constriction seen after local application of a vasoconstrictor drug, oxymetazoline (Neseril, Draco). In four experiments it was found that the decrease in gross pulse rate was 3–4 times greater following local application of oxymetazoline as compared with the increase seen on parasympathetic stimulation.

#### *Effects of atropine, bradykinin and indomethacin on secretion, tracer disappearance rate and blood content*

Intra-arterial infusion of atropine in doses (1 mg/kg) almost completely blocked the secretory response following parasympathetic stimulation in the range 0.5–20 imp/sec. However, atropine had no obvious effect on changes in tracer disappearance rate or gross pulse rate of  $^{131}\text{I}$  albumin. A representative experiment is shown in Fig 3.

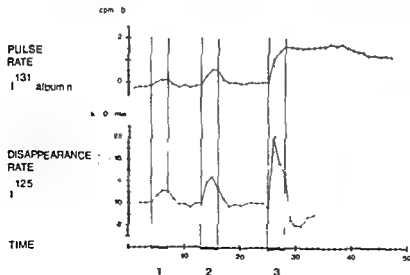


Fig 5 Influence of parasympathetic nerve stimulation and bradykinin on gross pulse rate and disappearance rate of  $^{131}\text{I}$  albumin measured over the right nasal cavity and disappearance rate of  $^{125}\text{I}$  albumin. Before starting the experiment secretion was blocked by 1 mg/kg Cat 3.5 kg (115: 8 V, 1 msec, 2.5 imp/sec). (2) Intra-arterial infusion of bradykinin 0.4  $\mu\text{g}/\text{min}$  and 0.4 imp/sec.

completely blocking secretion with atropine, intra-arterial infusion of bradykinin (0.1–1.0 µg/min) resulted in a considerable increase in disappearance rate. The maximal effects exceeded those seen after intra-arterial administration of papaverine and resembled the effects following nerve stimulation (Fig. 5).

To investigate whether or not prostaglandins might be involved as mediators of the vasodilatory responses, arachidonic acid (12 mg, at 0.5 mg/min), a prostaglandin precursor, infused intra-arterially in three experiments

no changes were noted on local blood content or disappearance rate. Furthermore, intra-arterial infusion of indomethacin (30 mg), an inhibitor of prostaglandin biosynthesis, had no effect on the pulse rate or disappearance rate following parasympathetic stimulation.

## DISCUSSION

In the present study stimulation of the parasympathetic fibres in the Vidian nerve resulted in increases in local blood content, secretion and disappearance rate of a water-soluble tracer, derived from the nasal mucosa, indicating a vasodilatory response as well as an enhanced tissue-to-blood exchange.

The rates of tracer disappearance have not been transformed into total blood flow values. Changes in capillary exchange surface area, capillary permeability, capillary flow distribution and velocity are of quantitative importance and influence the results (cf. Mellander & Johansson, 1968). The arteriovenous anastomoses within the nasal vascular bed, as described by Dawes & Prichard (1953), would also invalidate calculations of total blood flow based on  $k$ -values, since these are dependent on blood flow through the exchange vessels (Kety, 1960). For example, a redistribution of blood flow from shunt vessels to exchange vessels without changing total blood flow would increase the  $k$ -values. Furthermore, the simultaneously induced secretion interfered with the measurements and made quantitative evaluation unreliable. However, increases in disappearance rate indicate an increase in the tissue-to-blood exchange.

A vasodilatory response within the nasal mucosa in response to stimulation of the Vidian nerve has previously been demonstrated indirectly in rhinomanometric studies (Tschalussow, 1913; Blier, 1930; Malcomson, 1959; Malm, 1973). In these studies changes in the lumen of the nasal passages are recorded and the associated increase in secretion will affect the measurements (Drettner, 1963). With the present technique using  $^{125}\text{I}$ -labelled serum albumin this error is avoided and a frequency-dependent increase in the local blood content during parasympathetic stimulation was demonstrated. Furthermore, in contrast to most studies, a chronic sympathetic denervation had been performed, which prevented activation of the sympathetic vasomotor fibres in the Vidian nerve (Änggård & Densert, 1974).

It is probable that under physiological conditions all autonomic fibres have a low discharge rate, around 1–2 imp/sec (Folkow, 1955). At present, no information is available about the physiological parasympathetic discharge rate to the nasal mucosa. Eccles & Wilson (1973) studied nasal secretion during stimulation of parasympathetic fibres in the Vidian nerve without previous degeneration of the sympathetic fibres. Stimulation induced a frequency-dependent secretion with a minimal effective frequency of 2–5 imp/sec and a maximal secretion at 15 imp/sec. The present results, which show a noticeable secretion already at 0.5 imp/sec, seem to be at variance with those of Eccles & Wilson (1973). This can probably be attributed to the simultaneous activation of sympathetic vasoconstrictor fibres in their experiments. Thus, the present results suggest that the secretory responses are activated even at a low rate of parasympathetic discharge. This is further supported by the associated increases in tracer disappearance rate and local blood content, which indicate that the vascular and secretory responses in the nasal mucosa are activated simultaneously, in a frequency-response manner between 0.5–12 imp/sec.

Quantitative data on the relationship between vascular effects in the nasal mucosa following

parasympathetic and sympathetic nerve activation respectively are not available. In a previous study on the effects of sympathetic nerve activation (Änggård & Edwall, 1974) local application of a vasoconstrictor drug was found to result in a 80–90% reduction in local blood content, when compared with the maximal response obtained by sympathetic stimulation.

When the parasympathetic effects on local blood content are compared in a similar way with the maximal effects of sympathetic activation, as mimicked by local application of a vasoconstricting drug, the results indicate that local blood content and, indirectly, nasal patency is mainly influenced by changes in sympathetic tone. However, the profound increases in tracer disappearance rate following parasympathetic stimulation at frequencies below 1 imp/sec suggest a strong parasympathetic influence on the exchange function, as compared to the sympathetic effects (Änggård & Edwall, 1974).

Morphological studies on human nasal mucosa have demonstrated that the nasal glands and blood vessels are richly innervated by cholinergic axons (Ishii, 1970; Cauna et al., 1972). The results of morphological studies on cat nasal mucosa are in agreement with these findings (Änggård & Densert, 1974). Eccles & Wilson (1973) demonstrated that nasal secretion following stimulation of the Vidian nerve was blocked by activation of cholinergic receptors.

Such were highly sensitive to atropine, complete blockade was seen after as little as 5  $\mu$ g atropine/kg i.v. However, in the present study a slight secretion was occasionally observed at high frequency stimulation even after administration of atropine in amounts of 1 mg/kg. This might indicate that the present technique is more sensitive to slight changes in nasal secretion or that the results of Eccles and Wilson were affected by the simultaneous activation of sympathetic fibres as discussed above.

From the morphological studies showing a cholinergic innervation even around the blood vessels in the nasal mucosa it might be expected that the vascular responses to parasympathetic nerve stimulation would also be blocked by

atropine. However, the vascular response reflected by tracer disappearance rate and blood content, could not be blocked by atropine even though nasal secretion was completely abolished. This suggests a functional dissociation between the mechanism behind the activation of secretory and vascular responses in the nasal mucosa following parasympathetic stimulation.

Even if stimulation of the parasympathetic nerves to an organ increases the regional flow, this is no proof of the existence of vasodilator fibres, as the vasodilatation may equally well be induced by metabolites produced as a result of the increased tissue activity (Kow, 1955). Atropine resistant vasodilatation is well known from studies on salivary glands and has been attributed to the release of forming enzymes (Kallikrein) during activation of glandular elements, with a subsequent formation of vasodilating kinins (e.g. bradykinin) (cf. Hilton & Lewis, 1955). This was confirmed by Gautvik (1970) who further suggested that "all nerve fibres to the salivary glands are cholinergic and that the neuroeffector transmissions vary in their atropine sensitivity". Large numbers of glandular elements in the nasal mucosa and the present findings that secretory and vascular responses are activated at the same time might suggest a similar mechanism in the nasal mucosa.

Skinner & Webster (1968) suggested that atropine-resistant vasodilatation in the mandibular salivary gland was due to activation of  $\beta$  adrenergic receptors. This explanation is unlikely in the nasal mucosa since in the present study a vasodilatation occurred even after complete sympathectomy had been performed. Furthermore, in a previous study no evidence was found for the existence of  $\beta$  receptors in the nasal mucosa (Änggård & Edwall, 1974).

In the present study the effects of parasympathetic nerve activation resembled those of arterial infusion of bradykinin which suggests that kinins are also responsible for atropine-resistant vasodilatation in the

However, the vasodilatation might also be due to the release of vasoactive substances than bradykinin. The negative results obtained on stimulation or inhibition of the release of prostaglandins indicate that the dilatatory action is not due to the release of eicosanoids.

The present results suggest that following parasympathetic stimulation of the nasal mucosa sensory and vascular responses are activated simultaneously and within the same frequency.

The local blood content and thus intranasal patency, will only be moderately increased, while the tissue-blood exchange is increased even at a low parasympathetic discharge. The secretory responses are due to activation of cholinergic receptors, while the mechanism behind the activation of the vascular responses is still unknown.

## ZUSAMMENFASSUNG

Die Wirkung der selektiven parasympathischen Nervenstimulation auf die sekretorische Reaktion und auf das lokale Austausch- und Fassungsvermögen der Nasenschleimhaut wurde an der Katze untersucht. Dabei wurde eine lokale Abnahme von  $^{125}\text{I}$  und die Veränderungen der Abklingzeit unter Verwendung von  $^{125}\text{I}$ -markiertem Albumin in der Nase gemessen. Eine frequenzabhängige Steigerung der Nasensekretion und des lokalen Blutumschlages trat bei einer Frequenz von 0,5–12 Imp./sec auf. Gleichzeitig wurde eine Erhöhung der Blutmenge beobachtet. Diese Befunde deuten darauf hin, dass die vaskulären und sekretorischen Reaktionen zeitlich aktiviert werden. Die sekretorischen Reaktionen konnten durch Atropin gehemmt werden, nicht aber die vaskulären. Intraarterielle Bradykinin-Infusionen bewirkten eine Steigerung der Abklingzeit ähnlich der nach parasympathischer Nervenstimulation während Infusionen mit Arachidonsäure oder Indomethazin. Die Ergebnisse lassen erkennen, dass die postganglionäre parasympathische Überträgerstoff ist, wohingegen die Vasodilatation von einem Mechanismus durch Atropin oder Indomethazin nicht beeinflussten Mechanismus gesteuert wird.

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## ULTRASTRUCTURE OF THE EPITHELIUM IN ATROPHIC RHINITIS

### *Transmission Electron Microscopic Studies*

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(Received December 9, 1973)

**Abstract** Biopsies taken from various locations in the nasal mucosa of patients with atrophic rhinitis were investigated in the transmission electron microscope. Characteristic is a widespread metaplasia of the pseudostratified cylindrical epithelium to a keratinized squamous epithelium which corresponds very closely to thin skin. On the basis of the ultrastructural study a hypothesis concerning the pathogenesis of atrophic rhinitis is put forward. According to this theory an important feature in the development of some nasal symptoms is a decreased amount of secretory immunoglobulin A and consequently perhaps a reduced opsonising effect upon the bacteria in the secretion.

While the presence of a horny squamous epithelium on the outer surface of the body is useful protecting the organism against trauma and prevents dehydration, the presence of the same type of epithelium in the respiratory tract is unphysiological and may be harmful in causing symptoms and disease. It is known that chronic infectious reactions in the nasal mucosa can, to a certain degree, induce localized metaplasia of the ciliated epithelium to squamous epithelium (v. Dishoeck & Majer, 1964). In addition, it is known that space creating operations in the nasal cavity can evoke an atrophic rhinitis in the squamous epithelium, though most atrophic rhinitis has a quite unknown etiology and pathogenesis. However, Reichert & Hochstrasser (1971) recently demonstrated lack of a protease inhibitor in nasal secretion from patients with atrophic rhinitis. This may be an important factor in the pathogenesis of the disease, though

it is not known whether it is a primary or secondary phenomenon.

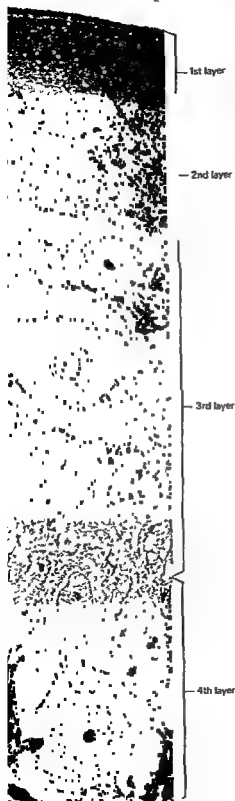
One assumption for an understanding of the causes of a disease is a knowledge of its etiology. In atrophic rhinitis Holopainen (1967) has extensively investigated and reviewed the literature. In electron microscopy, histochemistry and cytology, however, up to our knowledge no paper has been published about the transmission electron microscopy of this disease. Recently we presented a preliminary report on scanning electron microscopic studies of atrophic nasal mucosa (Mygind et al. 1974).

### MATERIAL AND METHODS

Six adult patients with pronounced atrophic rhinitis were examined. For a longer period of time they had all suffered from crusts in the nose, and they were only treated by conservative means.

Biopsies of a size of 1½-3 mm were taken with a small forceps without local anaesthesia. They were taken from the lower edge of the inferior turbinate ¼-1 cm behind the anterior edge, as from other places in the nasal cavity.

As glutaraldehyde renders the epithelium adherent to the forceps, the biopsies were removed from the forceps in 0.9% sodium chloride solution immediately after transferred to a cold solution of glutaraldehyde, buffered with a sodium cacodylate (pH 7.4). After 24



fixation the biopsies were stored in the buffer. The specimens were post-fixed for 2 hours at 4° with 2% osmium tetroxide, buffered with sodium cacodylate to pH 7.2-7.4. After dehydration in ethyl alcohol the blocks were embedded in Epon. Sections were cut in a Reichert ultra-microtome UM 2 with a Dupont diamond knife. Semithin sections were analysed in an optical contrast. Ultrathin sections were double stained with lead citrate and uranyl acetate and transmission electron micrographs were obtained with a Zeiss EM 9A electron microscope.

## RESULTS

A transitional epithelium as well as a horny squamous epithelium were seen in the biopsies. At the biopsied location in the front of the inferior turbinate a transitional epithelium is often found in normal persons. However, in a total of 100 biopsies from this site in normal persons and in patients with perennial rhinitis only four biopsies showed a keratinized epithelium (Mygind, 1974). Such an epithelium was found in 5 of 6 cases of atrophic rhinitis, and as a horny squamous epithelium therefore is characteristic of atrophic rhinitis, only this type of epithelium will be described (Fig 1).

The horny layer consists of 5-30 stratified keratinized cells with a thickness of 10-15  $\mu\text{m}$ , i.e. a total thickness of the horny layer of 10-30  $\mu\text{m}$ , on average. The keratinized cells are anuclear without cytoplasmic structures. They lie close together with short interdigitating structures, which at the surface correspond to the short

Fig 1 Low power view of transmission electron micrograph of the nasal epithelium in atrophic rhinitis ( $\times 3000$ ).

1st layer (Stratum corneum), consisting in this specimen of 12 layers of keratinized, anuclear cells, each layer with a thickness of 10  $\mu\text{m}$ .

2nd layer (Stratum granulosum). The cells are horn-

nucleolus

4th layer (Stratum basale). The vertically arranged cells, containing large nuclei, are in contact with the very thin basement membrane.

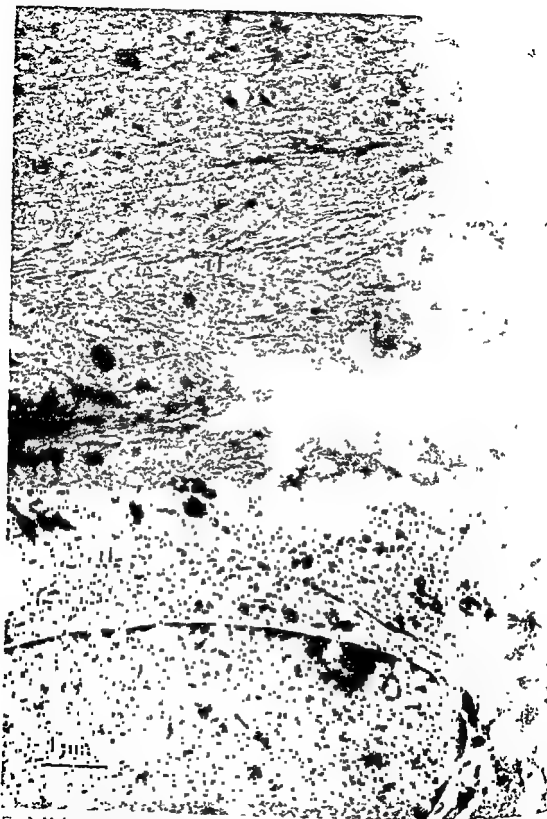


Fig 2. Medium-power micrograph of the 1st and 2nd layer of the nasal epithelium in patient with atrophic rhinitis. The keratinized cells are anuclear, without cytoplasmic structures. Short, interdigitating structures are seen, these are probably remnants of desmosomes. The

cells of the 2nd layer have elongated nuclei (*v*) with a fine chromatin pattern. The cytoplasm is dominated by horizontal tonofibrils (*tf*), keratohyalin granules (*g*), few mitochondria (*m*) and scattered polyribosomes ( $\times 17\,000$ ).



Some short, round mitochondria (*m*) and scattered ribosomes (*r*) are seen. Cytoplasm membrane (*cm*) with interdigitations ( $\times 30\,000$ )

riis (*l*), continuing as the desmosomes (arrow)

scanned (1974)

ists of  
 culated cells, it becomes apparent that the  
 bined structures probably are remnants of  
 psomes. The cells in this layer are spindle-  
 d with an elongated nucleus containing a  
 chromatin pattern. The cytoplasm is domi-  
 by several horizontal tonofibrils, some  
 ed kerato hyalin granules and a few mito-  
 ria. At different levels in this layer cells  
 zen with a more dark, condensed cyto-  
 a, representing a keratinizing process. In

the third layer (Fig. 3) the cells are irregularly  
 polygonal with a large round nucleus containing  
 a coarse chromatin pattern and 1-2 distinct  
 nucleoli. Also in these cells a dominant feature in  
 the cytoplasm is the concentrically arranged  
 tonofibrils which continue in the numerous des-  
 mosomes, which holds the cells together in colla-  
 boration with the folded cytoplasmic membrane.  
 Some mostly short, round mitochondria and  
 scattered polyribosomes are seen. The fourth and  
 lowest layer in the epithelium consist of verti-  
 cally arranged cells, which in survey magnifica-  
 tion have some resemblance to the basal

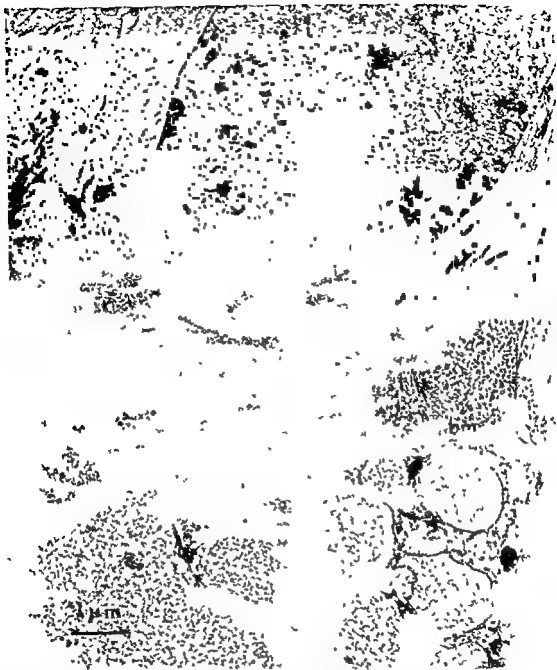


Fig 4 Transmission electron micrograph of the 4th layer of the nasal epithelium in patient with atrophic rhinitis. The cells are less firmly attached to each other, but do

contain some tonofibrils (*tf*). At the (*bm*) semidesmosomes (*sd*) are seen. The membrane (*cm*) is thin and locally absent. ( $\times 11$ )

the pseudostratified cylindrical epithelium. However, even these cells are characteristic of squamous epithelium in containing a moderate number of tonofibrils, which at the basement membrane sometimes form semidesmosomes (Fig 4). Especially in the upper part of the cells typical desmosomes are seen, whereas the lower part of

the cells are less firmly attached to the basement membrane. The cytoplasm also contains polyribosomes and mitochondria than does the superficial part of the epithelium.

The basement membrane is an electron-dense line, which is so thin that at low magnification it may appear interrupted between

magnifications disclose a continuous one. The connective membrane too is totally absent.

Islands in lamina propria were rare and glands were very seldom observed. When they were present, the epithelium of the upper part of it was of the same type as the surrounding stratified epithelium.

## DISCUSSION

Most of the light microscopic observations of Holopainen (1967) were confirmed in this ultrastructural study, i.e. the presence of islands of stratified epithelium, a considerable variation in thickness and type of the epithelium, a decreased number of glands and goblet cells and an absent or very thin connective membrane. We agree with Holopainen that the basement membrane is so thin that it is invisible in the light microscope, but the ultrastructural study revealed a continuous, thin membrane.

The resemblance to skin was striking in the stratified squamous epithelium with tonofibrils and desmosomes even in the cells in apposition to the basement membrane and with a horny layer of the same order of size as that of thin skin (Menton & Eisen, 1971; Odland & Reed, 1971).

This may imply that the atrophic epithelium, to a certain degree, has similar properties to skin-epithelium, i.e. impermeability for water.

The surface of the epithelium is therefore not moistened from the tissue fluid, and the number of glands is highly reduced. The epithelial surface necessarily becomes dry, i.e. metaplasia of the ciliated epithelium. The glands go down into the glandular ducts, the expulsion of secretion from these may be impaired, and cilia can assist in the extrusion of the secretion. This may be a pathogenic factor in the atrophy of the glands.

In normal skin does not permit penetration of immunoglobulin A (IgA) and does not synthesize secretory piece (Lai & Fat et al., 1973). In the case with the normal respiratory epithelium. Therefore, the concentration of secretory

IgA (two IgA molecules connected with one secretory piece molecule) in the atrophic mucosa may well be decreased in proportion to the area of metaplastic epithelium. Secretory IgA has probably an opsonising effect on the bacteria in facilitating their phagocytosis (Doe, 1972). This matter creates the basis for a theory, which could explain some features in the pathogenesis of atrophic rhinitis.

In patients with this disease the bacteria normally inhaled and filtered in the nose will not be caught by a continuous layer of mucus having a bactericidal effect and will not be transported to the rhinopharynx by the cilia, swallowed and destroyed by the gastric acid. They remain *in loco* and multiply, possibly with the dead horny cells as nourishment. The bacteria deliver factors chemotactic for neutrophilic leucocytes, which in large number penetrate the epithelium. On account of the lacking opsonising effect (due to the decreased concentration of secretory IgA) the leucocytes cannot phagocytize and destroy the bacteria, but are themselves attacked by bacterial products. The decreased leucocyte function brings about the constant production of chemotactic factors, which account for the continued penetration of neutrophils through the epithelium. In addition, the multitude of dying neutrophilic leucocytes release proteolytic enzymes in the secretion. These enzymes are not neutralized, due to lack of protease-inhibitor (Reichert & Hochstrasser, 1971). The free proteolytic enzymes in the secretion are probably harmful to the mucous membrane, as it is known that inhalation of proteolytic enzymes produces emphysema in animals (Johanson et al., 1973).

This theory can explain the following observation made by Holopainen: (1) The large amount of bacteria in the nasal smear, (2) the large number of neutrophilic leucocytes in the secretion compared with the lamina propria, where the mononuclear cells dominate, (3) the lack of intracellular phagocytized bacteria in the neutrophils in the secretion, (4) the decomposed appearance of these leucocytes.

The present ultrastructural study sheds no

light on the etiology of the disease, but shows that an important intermediate link in the pathogenesis is the factor or factors which influence the basal cells in the epithelium to synthesize tonofibrils and desmosomes. The fact is, that this feature causes the respiratory epithelium to metaplasize to squamous epithelium, since all cells are formed from the basal cells.

It is possible that the bacteria in the secretion, i.e. *klebsiella ozenae* (v. Dishoeck & Majer, 1964), are able to induce formation of tonofibrils in the basal cells, or such may be the case with immunological mechanisms provoked by the bacteria. Generally speaking, there is a total lack of information on the immunopathology of the nasal mucosa and on the content of antibodies in secretion in atrophic rhinitis.

## ZUSAMMENFASSUNG

Verschiedenen Stellen in der Nasenschleimhaut einiger Patienten mit atrophischer Rhinitis wurden Biopsien entnommen und im Transmissionsmikroskop untersucht. Charakteristisch ist eine weit verbreitete Metaplasie des mehrreihigen Zylinderepithels in ein der dünnen Haut sehr ähnliches keratinisiertes Plattenepithel. Aufgrund der ultrastrukturellen Untersuchung wird eine Hypothese um Pathogenese der atrophischen Rhinitis aufgestellt. Danach ist eine reduzierte Immunglobulin A Sekretion ein wichtiger Faktor für die Entwicklung einiger nasaler Symptome und vielleicht auch für eine reduzierte opsonisierende Wirkung auf die sich im Sekret befindlichen Bakterien.

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## SINOSCOPICAL BIOPSY IN MAXILLARY SINUSITIS

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111 consecutive sinoscopic biopsies from the mucosa of the maxillary sinus in patients suffering from chronic sinusitis were studied with regard to a number of histopathological parameters and compared with the appearance of the mucosa during sinusoscopy. No characteristic histopathological picture was found for either group of sinoscopic biopsies, but a statistically significant association was found between, on the one hand, thickening of the basal lamina, eosinophilia, loss of epithelium and an increased number of goblet cells and, on the other hand, the allergically dominated polypous and fibrous type of sinusitis. The reproducibility of the semiquantitative histological grading and the representativity of the biopsies were studied. The individual biopsies were not found to be representative with regard to all of the histopathological parameters. It is concluded that the introduction of a flexible biopsy forceps with optic system is necessary for the utilization of sinoscopic biopsy.

Histological studies of sinusitis have previously been confined to mucosa removed operatively from patients with chronic sinusitis and acute sinusitis with complications. Several differing histological classifications of sinusitis have been proposed on the basis of the above.

Acute sinusitis is most often classified as catarrhal or purulent based on the amount of inflammatory exudate, particularly polymorphonuclear leukocytes. Chronic sinusitis is classified by Jørgensen (1923) and Andersen (1943) into (1) an edematous or hyperplastic type, (2) an infiltrative or granulating type, (3) a fibrous type, (4) a mixed type.

The value of these classifications is limited by the fact that several histological types are frequently found in the same specimen of mucosa (Jørgensen, 1954; Davidson, 1969; Köhn, 1969). In addition Andersen (1943) and Schätzle (1963)

frequently found disagreement between the clinical and histological types of sinusitis.

Even though endoscopy of the maxillary sinus has been carried out in several centres since the beginning of this century, only a few studies have been published on mucosal biopsies removed by endoscopy. Bauer (1958, 1960) stated that there was good agreement between the sinoscopic examination and the microscopical appearance of a biopsy from the maxillary sinus in cases of allergic and infectious conditions.

We considered that it would be of interest to study how the changes, which are seen in biopsies taken from the mucous membrane of the maxillary sinus of patients suffering from sinusitis, correspond to the appearance of the mucosa during sinusoscopy.

In this respect we found that it would be necessary to investigate the validity of the histological grading and to what degree the single biopsy was representative of the mucosa as a whole.

### MATERIAL AND METHODS

The material consists of 157 biopsies originating from 200 consecutive attempts at biopsy taken from patients suffering from clinically and sinoscopically diagnosed maxillary sinusitis. In 43 cases either too little tissue had been removed or the tissue had suffered from too much trauma to be of use for histological examination.

111 of the biopsies suitable for examination



Table I *Reliability of the histological examination*

I Two independent gradings of 100 biopsies  
 II The grading of two separate areas on each of 50 specimens from surgically removed mucosa

	Per cent consistency		Difference
	I	II	
Basement membrane thickening	81	58	$\chi^2=9.01$ ( $p<0.005$ )
Oedema		48	
Fibrosis	76	72	$\chi^2=0.28$ ( $p>0.5$ )
Lymphocytes		60	
Plasma cells		72	
Polymorphonuclear neutrophils		52	
Eosinophilia	76	54	$\chi^2=7.49$ ( $p<0.01$ )

originated from sinuses with one of the 4 sinusoscopic pictures described below. The remaining biopsies originated from sinuses with a mixed sinusoscopic picture.

The sinusoscopic appearance of the mucosa in cases of sinusitis is divided into 4 types, as described by Illum & Jeppesen (1972), (1) an acute type with bright red, slightly swollen mucosa, (2) an oedematous type with pale, diffuse oedematous mucosa with a few dilated vessels, (3) a polypous type, dominated by multiple, pale polyps, and (4) a fibrous type, with irregular whitish solid areas between areas with oedema.

The biopsies were removed from a limited area on the rear lateral wall of the maxillary sinus using a fine cup biopsy forceps (Illum & Jeppesen, 1972). The tissue was fixed in formalin and cut into histological sections, which were stained using haematoxylin eosin, van Gieson's connective tissue stain and alcian green.

The histological changes were registered by one of the authors without him having any clinical information whatsoever. A semiquantitative grading was carried out 0-(+)-+ of some of the parameters: loss of epithelium, squamous cell metaplasia, loss of cilia and goblet cells, and 0-+-++-+++ of other parameters: mucus in epithelial cells, basement membrane thickening, oedema, fibrosis, lympho-

cytes, plasma cells, polymorphonuclear cells and eosinophilia.

The validity of the histological examination was investigated by grading the basement membrane thickening, fibrosis and eosinophilia in consecutive biopsies from the same patients 2-3 months after the first grading and comparing the results. The representativity of small area mucosa was evaluated by comparing the independent gradings of two fortuitous areas from sections from maxillary sinus mucosa operatively.

## RESULTS

*Reproducibility of the histological grading.* The semiquantitative grading of the 100 biopsies studied could be satisfactorily repeated 2-3 months after the first evaluation. A difference was found in 19% following grading of basement membrane thickening and 17% for fibrosis and eosinophilia of the biopsies (Table I). A difference of more than one grade was rare, however.

### *Representativity of a biopsy*

Table I also shows the results of the evaluation of two different areas of 50 specimens from maxillary sinus mucosa removed at operation, expressed as the frequency of occurrence of the parameters, which were graded. A  $\chi^2$  test shows a significant difference between the related gradings of the basement membrane thickening and eosinophilia, while this was not the case with fibrosis.

The difference between the basement membrane thickening and eosinophilia in two different areas of the mucosa thus cannot be explained by differences in the histological examination, but this can be the case with regard to fibrosis. A single biopsy cannot therefore be considered representative with regard to the first two parameters. Fibrosis appears to be more uniformly distributed throughout the mucosa.

### *The frequency of suitable biopsies*

Following 200 attempts at removal of suitable tissue was obtained in 157 cases.

## II Sinoscopic type of mucosal changes

	Acute type	Generalized oedema	Polypous type	Fibrous type	Significant difference
loss of epithelium					
+) - +	14/6	38/17	10/10	5/11	$p < 0.05$
goblet cell metaplasia					
+) - +	20/0	54/1	20/0	14/2	$ns$
goblet cells					
+) - +	16/4	42/13	10/10	12/4	$ns$
loss of cilia					
+) - +	8/12	34/21	8/12	3/13	$p < 0.05$
in epithelial cells					
+ / + + - + + +	12/8	28/27	9/11	10/6	$ns$
basal membrane thickening					
+ / + + - + + +	19/1/0	44/6/5	10/6/4	7/4/5	$p < 0.001$
lymphocytes					
+ / + + - + + +	4/5/11	5/21/29	0/5/15	2/6/8	$ns$
eosinophils					
+ / + + - + + +	17/2/1	47/6/2	18/2/0	14/0/2	$ns$
macrophages					
+ / + + / + + +	8/12/0	21/30/4	4/15/1	10/6/0	$ns$
plasma cells					
+ / + + / + + +	7/11/2	8/45/2	1/14/5	6/9/1	$p < 0.02$
polymorphonuclear leucocytes					
+ - + + / + + +	5/14/1	10/42/3	7/12/1	9/7/0	$ns$
eosinophilia					
+ + + / + + +	9/10/1	31/17/7	6/6/8	7/2/7	$p < 0.025$
(111 biopsies)	20	55	20	16	

the remaining patients there was either too little tissue or it had been subjected to so much trauma that it was unsuitable for histological examination. The size of the suitable biopsies varied from approx.  $1 \times 1$  to  $4 \times 6$  mm and was most often  $2 \times 3$  mm.

#### Comparison of histological and sinoscopic grades<sup>2</sup>

The biopsies were distributed between the sinoscopic groups as shown in Table II. The grading is detailed in this table on account of the statistical analysis. No characteristic histological difference was found for each of the sinoscopic types, but a  $\chi^2$  test carried out for each of the individual histological parameters showed, however, some differences between the groups. A significantly increased frequency of basal membrane thickening was found in the acute and polypous types ( $p < 0.001$ ). This also applied to eosinophilia ( $p < 0.025$ ). An increased frequency of loss of epithelium ( $p < 0.05$ ) and

loss of cilia ( $p < 0.05$ ) was also seen in the fibrous type. The polypous type was, in addition, dominated by plasmacellular infiltration ( $p < 0.02$ ) and goblet cells in the epithelium ( $p < 0.05$ ).

On the other hand no difference was found between the groups with regard to oedema and fibrosis, and there was no difference in the grading of the infiltration by lymphocytes and polymorphonuclear granulocytes.

#### DISCUSSION

The four most important factors that can interfere with the evaluation of histological changes are (1) bias, (2) reproducibility of the registered findings, (3) the representativity of a biopsy of the whole pathological process, and (4) the technical quality of the biopsy.

In the present study an attempt has been made to eliminate bias by carrying out the histological evaluation without having any knowledge of the clinical data. Bias that occurs owing to the presence or absence of other pathological changes could hardly be avoided.

The reliability of the semiquantitative

<sup>2</sup> We wish to thank H. Engberg-Pedersen, M.Sc., Leo Pharmaceutical Products, for performing the statistical tests.

tion used in the present study depends upon the reproducibility of the noted changes. This source of error is rarely studied in pathological investigations, but Ringsted & Ferebee (1964) found for example with double blind evaluation of 1104 sections from Kveim's test that 8% disagree when performed by one pathologist, while two pathologists were in disagreement on 21% of the sections. This agrees on the whole with our study, where a more detailed grading has been used, and where there was disagreement between one pathologist's evaluation when performed twice of 19-24% of 100 biopsies, depending upon the parameters studied.

The representativity of a single biopsy should actually be studied by independent evaluation of two biopsies from every sinus. As it was often impossible to obtain more than one biopsy with the technique used, it was necessary to evaluate this parameter by examining two areas of an operatively removed specimen. Single biopsies were found to be non representative with regard to thickening of the basement membrane and eosinophilia, while there was no demonstrable difference between places with regard to fibrosis. This does not correspond to the impression one has of the irregularity of fibrosis during sinuscopy. Kashiwado et al (1966) stated that a biopsy of mucosa was representative of the whole mucosa in 80% of the cases.

The quality of the biopsy is an even more important factor with regard to the histological examination. The average size and the frequency of successful biopsies are in agreement with Kashiwado et al (1966). Cup biopsies are, however, often somewhat compressed, particularly at the edges, which can result in the loss of oedematous tissue and the formation of fibrosis like artifacts. This can be the reason why no difference was found in the occurrence of oedema and fibrosis in the various sinusoscopic types of sinusitis.

Thickening of the basement membrane and eosinophilia were found significantly more frequently in biopsies from maxillary sinuses with fibrosis and polypous sinusoscopic pictures which are often seen in cases of prolonged allergy

Shambaugh (1931) and Weille (1930); a relationship between allergy and the basement membrane.

Loss of epithelium and cilia were frequently with the fibrous type and in agreement with Shambaugh (1931). A pronounced association between membrane thickening and loss of cilia. The increased occurrence of goblet cells in the polypous type is in agreement with (1963), who found an increased number of cells in the epithelium in allergic cases.

No significant difference was found in the occurrence of polymorphonuclear granulocytes in the 4 groups. This can possibly be due to the non uniform distribution of these cells in the submucosa, but also that they have more to do with inflammatory activity than to the extent of the inflammation (Lucas, 1952). (1943) found that polymorphonuclear cells are often absent even in cases of purulent sinusitis.

There was no difference either between the sinusoscopic groups with regard to the number of lymphocytes, while plasma cells were most frequently in the polypous type.

## CONCLUSION

There was thus only found a certain agreement between the macroscopical and histological appearance of the maxillary sinuses in cases of sinusitis. The most important possible causes of this is probably the considerable variation in the histological changes from one site to another in the mucosa, together with the fact that it is not possible to obtain representative biopsies under direct vision. Endoscopy will presumably be of most importance with regard to the study of localized processes in the maxillary sinuses and the introduction of a reliable biopsy forceps with optic is a prerequisite for further progress.

## ZUSAMMENFASSUNG

111 konsekutive sinusoskopische Biopsien der Kieferhöhlen Schleimhaut bei Sinusitis wurden blind und doppeltblind untersucht. Eine Reihe von histologischen Parametern

irden mit dem Bild der Schleimhaut bei Sinoskopie  
 ren  
 wurde kein charakteristisches histologisches Bild  
 Gruppe der sinoskopischen Bilder gefunden,  
 n aber ein statistisch signifikanter Zusammenhang  
 n einerseits Basalmembranenverdickung Eosino-  
 Epithel- und Zilienverlust sowie einer vermehrten  
 l Becherzellen und andererseits den von allergischen  
 dominierten polyposen und fibrosen Sinusitis-  
 n  
 Reproduzierbarkeit der semiquantitativen, histo-  
 en Gradierung und die Repräsentativität der  
 : n wurden untersucht. Die einzelne Biopsie war bei  
 histologischen Parametern nicht repräsentativ  
 ussfolgerung. Das Benutzen einer beweglichen  
 zange mit Optik ist für ein weiteres Ausnutzen der  
 opischen Biopsie erforderlich.

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## A METHOD FOR STANDARDIZED STUDIES OF MUCOCILIARY ACTIVITY

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(Received November 17, 1973)

**Abstract** A method for observing ciliary activity of mucous membrane is described and tested. Ordinary laboratory equipment is used. Variations of surface light reflections due to secretional wave movements are recorded under various conditions. The temperature and humidity parameters can be controlled. The method is standardized with regard to the following variable factors:

(1) intensity of light source, (2) optical and electrical magnification, (3) extent of area observed, and (4) recording speed. Rheological aspects of the mucociliary system will be discussed further in subsequent papers together with temperature and humidity effects.

There are two main approaches to the study of the mucociliary function of the upper respiratory tract. One is qualitative observation of the transportation rate of small particles (Sharpey, 1835, Hill, 1928, Hilding, 1932, Messerklinger, 1951, Dahm, 1956, Ewert, 1965) or quantitative observation of the removal of a known amount of deposited or inhaled particles, i.e. clearance estimations (Baetjer & Bates, 1966, Baetjer, 1967, Rylander, 1968, Quinlan et al., 1969). The other is to record the beat frequency of the cilia or, more correctly, the frequency of mucous waves brought about by the beating cilia. The latter approach was used as early as 1884 when Marius estimated the ciliary beat frequency with a stroboscope. The stroboscopic technique has been further developed by Gray (1931), Ballenger & Orr (1963) and by Andersen (1971). Photographic techniques were introduced by Gray (1931), Lucas (1931), Proetz (1932), Frenckner & Richt-

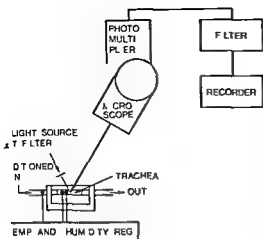
ner (1939) and by Dalhamn (1956). By using a photocell with a focusing screen, TV screen display were published by Dalhamn & Rylander (1962), Håkansson & Toremalm (1965), Guillemin et al. (1965) and by Toremalm (1967). Chevanec & Lennon (1970) described a microphoto-oscillographic method in which a photomultiplier

Stroboscopic methods do not permit infrequent frequency changes to be followed and oscillographic methods are time-consuming and expensive for routine experiments where immediate results are wanted. The modern methods using photocells or photomultipliers seem to be the most suitable for this type of experiment.

Respiratory tract mucosa is preferred for experimental tests regarding the effect of various substances and air pollution materials on mucociliary function (Negus, 1934, Proetz, 1933, Dalhamn, 1956, Krueger & Smith, 1958, Dahm, 1956, 1972). The problem is, however, that the methods and types of equipment are so different that it is impossible to compare in detail the results from different research groups. In our opinion there is an urgent need for a relatively simple method for ciliary function studies, which can be easily reproduced in most experimental and clinical laboratories.

The aim of the present investigation was to design and test a method based on our own experimental experiences (Håkansson & Toremalm, 1965, 1966 and b, 1967, 1968) and meeting the following requirements:

This investigation has been supported by grants from the Swedish Medical Research Council (Project number B73-14X-3897-01).



Block diagram of the experimental equipment (see

instantaneous recording of wave movements  
the secretion layer in experiments of long  
duration

sensitivity to rapid frequency changes  
uncomplicated analysis of the recordings  
simultaneous recording and regulation of the  
temperature and humidity parameters  
use of routine laboratory apparatus

## METHOD

The experimental arrangement consists of (1) a  
light source (2) an experimental chamber for  
the specimens (3) a binocular microscope  
(4) a photomultiplier (5) a frequency filter and  
an ink writer as seen in the block diagram  
fig 1 The laboratory set up is shown in  
fig 2

The light source is a fibre optic system  
clinically used for endoscopy The appa-  
ratus has five ordinary grades of light intensity  
in a relation 1:2:3:5:4:2:6:5 There is no  
measurable heat gradient near the mucous mem-  
brane between the minimum and maximum  
intensities The spectrum of the light source  
measured in the microscope ocular is 400–850

A perspex experimental chamber according to  
Kansson & Toremalm (1965) is used It is  
double walled for insulation purposes and sup-

plied with known amounts of tempered and  
humidified air from a thermostat The conditioned  
air is blown through the trachea where the  
temperature is measured by thermocouples The  
present experiments have been performed at  
temperatures between 20° and 37°C and a rela-  
tive humidity above 90%.

The binocular stereomicroscope or operation  
microscope (Zeiss) is modified so that it can be  
manoeuvred in all directions This makes it  
easy to focus a suitable spot on the mucous  
membrane preparation Two special oculars  
(Zeiss 25×) are used One has a special grid  
for exact identification of object position A  
diaphragm is mounted in the other ocular This  
consists of a disc with a central opening of 1.1  
mm diameter Several discs with other apertures  
have also been tested The hole must be positioned  
exactly in the centre of the optical axis The edge  
of the hole is bevelled at that side of the disc  
which points away from the observer's eye in  
order to avoid local reflections which may inter-  
fere with the original ones The area to be ob-  
served is first identified and focused in the  
centre of the grid Then the grid ocular is re-  
moved and the diaphragm ocular is put in place  
On top of this second ocular a photomultiplier  
(EMI 9524 B) is mounted It is fed by 1 000 V  
d.c. via a power supply (Oltrox A 2.5 K 10  
HR) A total optical magnification of 100× is  
used

A frequency clipping filter (Krone Hite type  
3550) is used in order to exclude occasional  
disturbing frequencies e.g. from the electric  
mains Frequencies below 3 Hz and above 30  
Hz are eliminated

A direct writing recorder (Elema Mingograph  
34) is used for recording of the light reflection  
variations It has a frequency range of 0 to 700  
Hz The paper speed has been either 25 or 100  
mm/sec Normally a 1 minute section of the  
record is analysed The noise level of the ampli-  
fication system was measured to 200 μV The  
signals obtained are 24 dB above this level.

For the actual experiments the rabbit trachea  
is mounted in the experimental chamber imme-  
diately after the animal has been killed by a blow

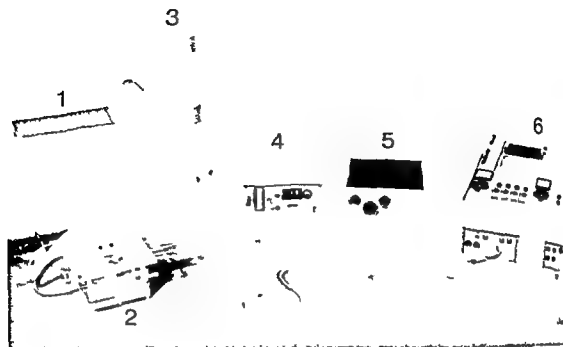


Fig 2 Details of the experimental equipment 1 light source 2 experimental chamber 3 microscope and

photomultiplier 4 magnifier 5 frequency divider

on the head in order to avoid the influence of anaesthetic drugs. Rabbits with a weight of 1.5–2 kg have been used.

All experiments have been made on a non-rating table in an electrically shielded room.

## RESULTS

The present equipment for indirect observation of the ciliary activity of respiratory epithelium includes different optical, electrical and mechanical details. The capacity of each is basically tested by the manufacturers. When combined into a system they need to be trimmed in order to give maximum efficiency and a minimum of undesired disturbances. Some parameters of the experimental situation are kept constant, e.g. the choice of experimental animal, preparation technique, environmental temperature and humidity. Other parameters have been experimentally tested, i.e. (1) intensity of the light source, (2) magnification of the microscope, (3) the extent of the supervised area regulated by various

diaphragms in front of the photomultiplier, (4) magnification of the photomultiplier and (5) the speed of the recorder.

The experiments with increasing light intensity are demonstrated in Fig 3. Five different degrees of intensity have been tested. In the figure the light intensities in grades 3, 4 and 5 are adequate since the recordings can be easily analysed. Grade 1 as a routine light intensity.

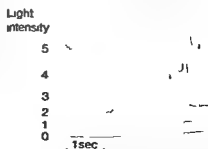


Fig 3 Light intensity and amplitude variations are made from the same track at different light intensities. Temp. 27.6°C. Humidity >90%. Diaphragm diameter 0.1 mm. Speed 100 mm/sec.

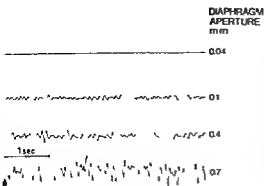


Fig. 4 The relationship between supervised area (regulated by different diaphragm apertures in front of one eye) and the recorded secretion wave patterns. Temp 33.2°C. Relative humidity >90%. Light intensity = 3. Recording speed 100 mm/sec.

Different degrees of magnification have been used and a total optical magnification of 100× proved to be suitable for ciliary function studies as the wave pattern can easily be identified.

It is necessary to focus an area wide enough to represent the wave movements initiated by a group of coordinated ciliary cells and small enough to exclude too many interchanging wave movements. The choice of diaphragm is therefore of significant importance. In order to find a suitable observation area four different discs were used with round central openings of 0.04, 0.1, 0.4 and 0.7 mm diameter respectively. The results are demonstrated in Fig. 4. If the opening is too small no light reflection changes can be detected (Fig. 4 upper). If the opening is too large, however, too many different waves are recorded which complicates the interpretation of the findings (Fig. 4 lower). An opening diameter of 1 mm gives an uncomplicated recording.

Table I

Diaphragm aperture (mm)	Square area ( $\mu\text{m}^2$ )	Ciliary cells (approx.)	Cilia (approx.)
0.04	22 690	600	145 000
0.1	3 320	90	21 000
0.4	380	10	2 400
0.7	79	2-3	500-700

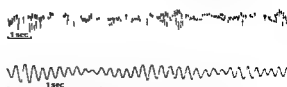


Fig. 5 Original record from the rabbit trachea at a temp of 33.2°C and relative humidity >90%. Light intensity = 3. Diaphragm diameter 1 mm. Paper speed for routine follow up = 25 mm/sec (upper). Paper speed for analysis and calculation = 100 mm/sec (lower).

from which the frequency can easily be calculated. This means that a square area of about 380  $\mu\text{m}^2$  is analysed. An area of this dimension covers approximately 10 ciliary cells comprising about 2 400 cilia (see Table I). With the use of this "ideal" observation area, rhythmic amplitude and frequency changes are seen (Fig. 5).

The sensitivity curve of the photomultiplier is well within the range of the frequency spectrum of the light source. The power supply of the photomultiplier was 1000 V during the entire experimental series.

The standard paper speed for all the experiments is 25 mm/sec (Fig. 5 upper). To facilitate the determination of frequency, a speed of 100 mm/sec is recommended (Fig. 5 lower).

Fig. 6 shows the effect on the record, when the light source is switched off. This manoeuvre is used for calibration purposes.

## DISCUSSION

The study of the mucociliary system of the respiratory tract is of growing interest in view of increasing local and personal air pollution and the effect of pharmacological agents, e.g. mucolytic drugs, which are applied directly to the mucous membranes. The interest of such studies has led to the development of numerous methods and



Fig. 6 Test to check for mechanical, optical and electrical disturbances. Temp 38.0°C. Relative humidity >90%. Light intensity before and after test = 3. Recording speed 25 mm/sec.



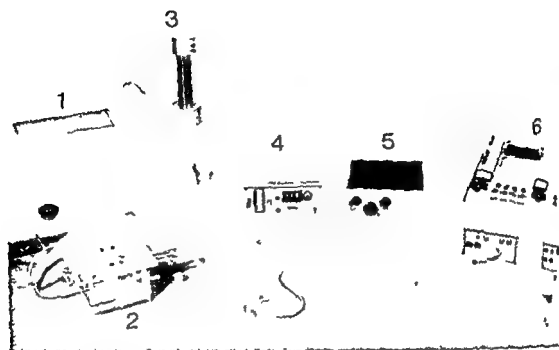


Fig. 2. Details of the experimental equipment: 1 light source, 2 experimental chamber, 3 microscope and

photomultiplier, 4 magnifier, 5 frequency counter and recorder.

on the head in order to avoid the influence of anaesthetic drugs. Rabbits with a weight of 1.5–2 kg have been used.

All experiments have been made on a non-brating table in an electrically shielded room.

## RESULTS

The present equipment for indirect observation of the ciliary activity of respiratory epithelium includes different optical, electrical and mechanical details. The capacity of each is basically tested by the manufacturers. When combined into a system they need to be trimmed in order to give maximum efficiency and a minimum of undesired disturbances. Some parameters of the experimental situation are kept constant, e.g. the choice of experimental animal, preparation technique, environmental temperature and humidity. Other parameters have been experimentally tested, i.e. (1) intensity of the light source, (2) magnification of the microscope, (3) the extent of the supervised area regulated by various

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The experiments with increasing light intensity are demonstrated in Fig. 3. Five degrees of intensity have been tested. In the figure the light intensities in grades 3, 4 and 5 are adequate since the recordings can be easily analysed. Grade 1 as a routine light intensity.

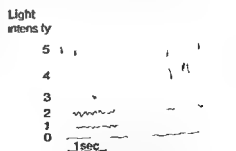


Fig. 3. Light intensity and amplitude variations. Recordings are made from the same tracheal cilia at different light intensities. Temp. 27.6°C, Humidity >90%. Diaphragm diameter 0.1 mm, recording speed 100 mm/sec.



# FOLDING MECHANISM OF THE HUMAN LARYNX

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(Received November 5 1973)

**Abstract** The mechanism of the human larynx often regarded as sphincteric more nearly represents graduated folding or plication Taking the end of normal expiration as the reference condition folding decreases with inspiration and increases successively with reserve expiration phonation effort closure and swallow closure

When fully closed the human larynx can successfully resist the greatest efforts of the body to blow it open The mechanism of this hermetic closure puzzled many early observers Dodart (1700) had called the glottis a rectilinear sphincter capable of narrowing without shortening Negus (1929) proposed that the striated muscles closing the larynx were components of a sphincter

phylogenetically descended from a circular sphincter closing the entrance to the primitive larynx Doubt about this derivation arose from the fact that in Protopterus, the South African lung fish ostensibly a representative of the primitive state, the sphincter consists of smooth muscle (Goepfert, 1937) Certainly, in the human larynx, the anatomical arrangement of the striated muscles is not that of an ordinary sphincter A different and persuasive explanation incriminating check valve action emerged about a hundred years ago from the studies of Wyllie (1866), Lauder-Brunton & Cash (1883), and others, but when X ray investigation was brought to bear on the problem, no sign of a check valve could be detected (Lindsay, 1940) At present the true nature of the closing mechanism remains ill defined Pressman (1944) proposed a

combined sphincteric check valve mechanism but this is opposed by an increasing amount of X-ray evidence (Fink, 1956, Ardran & 1967) and is either in abeyance or unrecognized for want of a clear alternative (1972)

Interestingly enough the elements for interpretation have long been at hand The like arrangement of soft tissues in the larynx was pointed out by Bertin in 1745 and discussed by the anatomist Henle at least as early as 1866 (Henle, 1866) The Basle Anatomica (His, 1895) named three such folds: plica vocalis, plica vestibularis, plica aryepiglottica Several developments make it reasonable to propose that plication—the formation and maintenance of folds—rather than sphincterization is the mechanism of the main changes in the configuration of the human larynx

## *Objections to the sphincteric theory*

A sphincter as defined in dictionaries (e.g. English Dictionary, Larousse) signifies a ring or annulus of muscle that normally produces constriction of a bodily passage In the human body muscle sphincters of the esophagus and rectum are most intimately related to the larynx They form a sheath to the canal and have numerous attachments In the smooth muscle sphincters of the stomach, the bladder and the biliary ducts the arrangement of fibers is circular and these descriptions fits the morphological characteristics of the intrinsic muscles in the human larynx

This work was supported by USPHS Research Center Grant GM 15991

unctionally, the orifice guarded by a sphincter opens briefly when material is pushed through passage during relaxation of the sphincter the mammalian larynx it is closure of the sage that is momentary, and most of the the passage is open. Moreover the open e in the human can be at least partly maintained without a dilator muscle, as is seen during natory closure of the glottis, when abduction he arytenoid cartilages is inoperative yet the ibule remains open.

In sum, irrespective of phylogenetic origins, striated musculature of the larynx in man longer has the morphological or physiological ibutes of a true sphincter, and produces a ncteric closure only in quite a loose sense he term. I now attempt to show that folding nearly describes the sequence of events.

#### *Active features of the laryngeal mechanism*

These may be considered under four headings: inspiration, phonation, effort closure (coughing, effort, defecation, and the like) and swallow ure, representing the four major patterns of ivior into which the human larynx is organ-

At the outset it is appropriate to stress Kotby & Haugen (1970a) that the laryngeal hanism is highly complex and that individual ations in the role of particular components to be expected.

#### *Inspiration*

In the respiratory cycle vertical movements take place in the diaphragm and in the train of cartilages comprising the trachea and larynx. Inspiration leads to descent of the trachea and stretching of the laryngeal soft tissues near the hyoid. The vestibular folds and aryepiglottic folds and the preepiglottic body become elongated vertically and thinner transversely (Figs 1B and 2A) in association with increased separation of the hyoid bone from the thyroid and arytenoid cartilages. Viewed as a whole these changes may be said to represent a process of folding. The arytenoids, constrained by the arytenoid articulation to travel downward

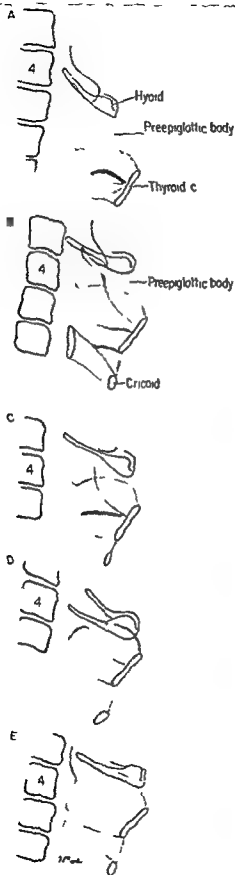
as well as outward, eventually rock backward (Fink et al., 1956, Ardran & Kemp, 1967), apparently obedient to the tension in the folds. The effect of this is to flatten the vocal folds against the side wall, a further unfolding. Expiratory ascent reverses the displacements (Figs 1B and 2B) that is to say it produces partial folding aided by recoil of the elastic tissue stretched in the descent (Fink, 1974b).

The role of the crico-arytenoid muscles in this cycle has been much debated. Current evidence seems to favor the view that in man the muscles stabilize the upright posture of the arytenoids rather than directly adduct or abduct these cartilages (Kotby & Haugen, 1970b). Accordingly at the end of normal expiration the arrangement of the laryngeal structures in part reflects an equilibrium between passive forces and may conveniently be taken as the reference condition. Inspiration then represents an active decrease in laryngeal plication, reserve expiration an active increase, linked respectively to the active augmentation and the active reduction of lung volume.

In general, the larynx and the lungs are constantly varying in shape and size with respiration, their variations being coupled neurally through the respiratory center and mechanically through the trachea. Part of the significance of plicatory coupling evidently resides in the fact that it relates the changes in volume and air flow resistance of the laryngeal air space to the changes in volume and flow resistance of the pulmonary air space.

#### *Phonation*

Phonatory adduction of the arytenoid cartilages draws the posterior attachment of the vocal folds to the midline. The adduction of the arytenoids is facilitated by the presence of the pyriform fossa but the associated excursion of the vocal folds is constrained by the thyroid cartilage. Molding of the folds by contraction of the vocalis muscle is facilitated by the concomitant partial closure of the crico-thyroid angle or "visor", which in effect makes vocal fold tissue available for redistribution toward the midline. The result



may be reasonably described as increase of the vocal fold

Folding of the vestibular folds increases in phonation, these folds are medially passively with the arytenoids at same time pulled slightly upward by tension in the aryepiglottic folds which tends to enlarge the ventricle (F) and provides space for a large amplitude vibration of the vocal folds (Muller, 1941; 1962). Additional laryngeal folding does at the level of the preepiglottic body (epitubercle, see below) with many vocal tremants as a result of vertical movements of the hyoid bone and larynx (Perkell, 1969).

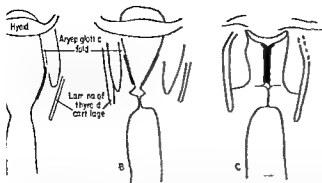
### Effort closure

In efforts such as coughing, defecation, or a heavy weight, a temporary hermetic seal of the larynx develops in which plication of the vestibular and aryepiglottic folds is added to that of the vocal folds.

The adduction and plication of the vocal fold is thought to be effected by the thyroarytenoid muscle, which has fibers running obliquely upward and backward, and draws the vocal fold not only medially but also downward, thus obliterates the ventricle and apposes the vocal folds to the adducted vocal folds.

The aryepiglottic fold, containing the end of the cuneiform cartilage is also retracted during effort closure. It folds along the lateral margin of the cuneiform (Figs 1B, 2C), which was called the point flexion of the aryepiglottic fold by Stuart (1892), so that

Fig 1 Idealized sketches based on X-ray illustrating the folding (plicative) nature of laryngeal mechanics. Lateral view. (A) In rest, the aryepiglottic, vestibular and vocal folds and preepiglottic body elongated and flattened. (B) Expansion of the aryepiglottic, vestibular, and vocal folds and preepiglottic body somewhat shortened. (C) Phonation—partial plication of vocal fold, partial plication of preepiglottic body. (D) Vestibular fold separated from the vocal fold, opening the ventricle. (E) Effort closure—marked plication of preepiglottic body, plication of the epiglottic cartilage at the base of the epiglottis.



2 Idealized sketches based on tracings of anterior tomograms of the human larynx. The position of cuneiform cartilages (black bars) is inferred from eye observations with a Macintosh laryngoscope. **Deep inspiration**—Aryepiglottic folds elongated and flattened against the thyroid cartilage lamina. (B) **Deep expiration**—Ary-

epiglottic folds shorter, vestibular and vocal folds thicker representing the reference condition of partial plication. (C) **Effort closure**—Plication of aryepiglottic, vestibular and vocal folds. Cuneiform cartilages in apposition. Ventricle almost obliterated. The apparent angle of divergence of the thyroid cartilage laminae (angle of flare) decreases slightly from A to C.

form moves medially to the midline while the rest of the aryepiglottic fold is directed laterally, applied to the back of the epiglottic lamina. Since the aryepiglottic fold indirectly links the thyroid cartilage to the hyoid, its folding or plication is probably facilitated by approximation of the thyroid to the hyoid, much as plication of the vocal folds is aided by approximation of the thyroid to the hyoid. Approximation of the thyroid and hyoid is an integral part of effort closure of the larynx and also causes the tubercle of the epiglottic cartilage to bulge backward a little (Czerwik, 1861, Fink, 1956) and contact the plicated vestibular and aryepiglottic folds. The midline apposition of the cuneiforms involves strain of the tissue joining the cuneiform to the arytenoid lamina, and the elasticity of this tissue assures that on release of the strain the cuneiform automatically returns to its lateral position. In this way the cuneiform-arytenoid zone of strain is one of the many springs that secure the open configuration of the human larynx (Fink, 1973a).

#### Swallow closure

Two types of swallow closure of the larynx have been identified (Ramsey et al., 1955), one used in drinking a long draught without pause by "gurgling a drink down one's throat", during

which the larynx remains low and the epiglottis upright, the other involving elevation of the larynx, forward propulsion of the hyoid, and retroversion of the epiglottis. In both, approximation of the larynx and hyoid distorts the pre-epiglottic body, causing the lower part of the epiglottic cartilage to bulge backward as noted above under effort closure. In addition, in the complete swallowing act the epiglottis often folds backward and downward and undergoes a final plication that caps the other plications already present lower in the larynx (Fig. 1E).

#### Rationale of plication

Several features of the human laryngeal mechanism are easier to understand as plication than as sphincteric action.

That the gradations of closure in expiration, phonation, effort closure, swallow closure, are better described as graduated folding than as graduated sphincteric closure emerges from consideration of the closure of swallowing and its tier of four foldings, two antero-posterior ones, the vocal and vestibular, surmounted by a T folding—the aryepiglottic—and a transverse folding, the epiglottic. Crossing of plications at the T clearly increases the security of closure, but eludes interpretation in a sphincteric scheme. Indeed the two upper stages are scarcely sphinc-

teric even in a broad sense, but need no qualification as foldings, accumulated successively in effort and in swallow closures

Sphincteric closure encompasses only the actions of the small, intrinsic muscles of the larynx, but folding takes into account the effects both of extrinsic and intrinsic muscles and unifies them into a single mechanically coordinated system. For example, the elevation of the larynx during swallowing is understandable in part as a vertical shortening necessary for completion of the four-tier plication, as a condition of complete sphincteric closure the elevation lacks rationale. Similarly, the vertical rhythm of the trachea during respiration can mechanically couple to folding and unfolding of the laryngeal folds, but not to sphincteric rhythm of the folds. Finally, a newly described sagittal pliability in the larynx (Fink, 1973b) essentially a slight folding and unfolding of the thyroid cartilage at the isthmus, attributable to extrinsic muscle action is readily understood as integrated with the vertical movements of the larynx but has no apparent connection with sphincteric action.

Last, but not least, the term "plication" possesses a useful semantic attribute. Where disorders of sphincteric closure tend to be thought of as disorders of neurological control, disorders of plication suggest two kinds of possibilities, disturbances of control and disturbances of a purely mechanical order, a distinction that is of potential clinical significance.

## ZUSAMMENFASSUNG

Der Mechanismus des menschlichen Kehlkopfs, der oft als sphinkterisch betrachtet wird, stellt eher einen differenzierten Faltungsprozess dar. Wenn man das Ende der normalen Ausatmung als Bezugsbedingung ansieht, so findet man, dass die Faltung mit der Einatmung abnimmt und bei reservierter Ausatmung Phonation. Kehlkopfschluss bei Anstrengung und Kehlkopfschluss beim Schlucken stufenweise zunimmt.

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## MICRODYNAMICS IN VOCAL FOLD VIBRATION

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(Received December 13, 1973)

The question what causes damage to the vocal in habitual faulty use of the vocal organs, is and in part by the morphological characteristics of the folds. Experiments with a large stress exerted on the ligaments do not clearly support the hypothesis rupture of collagen fibres may occur in vocal strain.

Larynx when functioning in phonation can be exposed to considerable stress. Two forces contribute to this stress: the air stream and the muscle contractions. The total mechanical power of the air stream is estimated as being approximately  $\frac{1}{2}$  watt (Salmon, 1964). Only a small part (0.5 to 0.5%) of it is transformed into acoustic energy. The remaining energy is transferred to the air stream and the vocal folds. The question arises, whether the larynx is able to endure the influence of the transmitted energy, or it may perhaps be traumatizing. Are the acting forces of the laryngeal muscles big enough to cause trauma to the vocal fold tissues? The aim of the present article is to survey this question, paying special attention to the connective tissue.

The transfer of energy from the air stream to the vocal fold can be illustrated easily by the following experiment. A transparent finger of a surgical glove is filled with water in which pieces of paper are suspended. This "vocal" is pressed against a hole in a transparent plate. When the pressure is adequate an air stream directed through the hole will result in vibrations of the model and a clear voice can be heard. At the same time strong rotatory and

turbulent movements of the pieces of the paper inside the model can be observed. It is conceivable that such movements would occur in the actual vocal fold if it were composed homogeneous, as is the water in our experiment. In the vocal folds however the inner movements are considerably damped, mainly by the connective tissue which makes up about half of the contents of a vocal fold. In the connective tissue collagen and elastic fibres form a matrix in a very viscous solution. The special arrangement of these fibres modifies and restrains the tissue movements inside the vocal folds. Certain parts of the connective tissue, e.g. the middle part of the vocal ligament, are more exposed to the effects of whirling vibration energy than are other parts. If the quantity of absorbed energy is too large, traumatization of the connective tissue may occur.

We have asked ourselves the following questions: How strong is the resistance of the connective tissue of the vocal folds to mechanical trauma? Of what magnitude are the forces acting on the ligaments? To what extent is the anatomical construction of the vocal fold related with these forces?

Connective tissue in general is composed of connective tissue cells and of extracellular fibres (Bloom & Fawcett, 1968). They are embedded in an amorphous substance containing tissue fluid. There are at least three types of extracellular fibre: collagenous, and more and less elastic fibres, in parallel and in reticular formation. Recent evidence indicates that collagenous and



reticular fibres may simply be different morphological expressions of the same fibrous protein (Gould, 1968). The chemical composition and physical properties are relatively well known. It is generally accepted that the primary function of collagen fibres is a mechanical one, that is, to resist tension along their axis (Harkness, 1968). The orientation of the fibrils would be in response to mechanical stress and to cellular influences such as geometric necessity (Holm, 1963). Collagen is about as strong as the strongest known animal or plant fibres (Morgan, 1963). Its extensibility is less than 5% in the normal range of tension, when the fibres are parallel, but up to about 30% in the case of fibres forming feltworks. Tendons contain about 30% of collagen on a wet weight basis. The ultimate strength of mammalian tendon subjected to longitudinal tension is from 5 to 10 kg/mm<sup>2</sup> or, in terms of collagen, 15 to 30 kg/mm<sup>2</sup>. According to Harkness it seems likely that the true strength of the collagen is that found in the smallest fibres, the larger ones consisting of aggregates of smaller ones, which are not ideally arranged and thus do not share the load equally. Consequently, some can be broken before others, with a net reduction in strength. With increasing stress the tissue becomes increasingly less extensible. The early part of this change in mechanical properties is associated with a visible straightening of initially wavy collagen bundles (Harkness, 1968). The tensile strength rises during growth to a plateau and later falls in old age (Röllhäuser, 1950, Stucke, 1950, Yamada, 1964). Elastin differs in some respects from collagen with which Harkness found that elastin in its pure form can be extended to twice its resting length. In elastic fibres breaking occurs when they are stretched to about 150% of their original length. This requires a force of only 0.2–0.3 kg/mm<sup>2</sup>.

Shrinkage by heat is another property of interest for a hypothesis on vocal nodules. The shrinkage temperature varies with the different collagens. The low values of 55° to 61°C were obtained from a 34-week-old foetus and the highest values, 63° to 67°C, from a 61-year old

man (in fascia lata) (Brown et al., 1957). Shrinkage temperature in water for the collagens is 24° to 29°C above the denaturation temperature (Gould, 1968).

#### *Arrangements against Traumatization Caused by Internal Friction (Heat)*

Lymphatic and blood circulation can be said to prevent the excessive heat production in the vocal folds. Our knowledge of this different voice habits is, however, very limited. Basic studies are still lacking.

In the ligaments and tendons the elastic fibres form a network. The spaces between the elastic fibres are filled with a delicate feltwork of collagenous fibres and a few fibroblasts. In the interfibrillar spaces there is a colloidal material consisting of polysaccharides and hyaluronic acid which, by binding varying amounts of water, can change its volume. If it were not for the interfibrillar material, the tissue would be extensible until the fibres were fully stretched. Now the interfibrillar mass offers resistance to the deformation of the interfibrillar spaces. When, owing to a decrease of volume, the spaces between the fibrous partitions are pressed together, the polysaccharides being of comparatively small molecular weight, are of great importance for the water binding.

Hyaluronic acid, however, can retain a large amount of water up to 2 000 times its own weight. When it is in hydrodynamic equilibrium, a hyaluronic acid molecule is a long, thin, thread-like molecule which, when not subjected to an external force, will curl to a random coil. It can be distended to an almost infinite length but when left to itself will immediately contract into a ball. This is due to the mutual attraction of the water ions with which the hydrophobic coil are clad. For this reason hyaluronic acid is the ideal physiological greasing substance. It fills any space between moving surfaces, thus impeding the movements by friction. This has been demonstrated for the articulation of joints and for the umbilical cord. The fibrous lamellar surfaces are covered by a monomolecular layer of hyaluronic acid.

anism is likely to be met in any situation ■ one layer ■ sliding loosely over the other, the gut, the skin, and also in the subepithelial layer of the vocal folds and vocal ligaments. There are several phenomena in connection with the human voice which may have to do with chemical and physical properties.

• Every voice needs a period of warming up before it can perform its full range of pitch and volume. If the warming up period is omitted, high intensity and pitch are only attained by straining, and damage to the vocal fold may result. Warming up period, e.g. in singing, is often explained in terms of adaptation of the blood circulation by work. Local blood flow reflexes are, however, so quick that a steady state would be reached sooner. Thus they do not account for this phenomenon. We are inclined to believe that slight chemical and physiological changes have to take place, e.g. a shift in water content of the connective tissue and in fibrillar spaces before the organ is under voluntary control.

• Edema of the subepithelial layer is a common consequence of vocal misuse. This is characterized by a shift of water content and one that takes days or months to be restored to normal. The degree of succulency is apparently required for producing the full scale of vocal possibilities. Excess of fluid impairs voice production, however.

• When a woman's voice is feminized by oestrogenic hormones, there are no signs of edema of the subepithelium. Possibly there is a permanent change in the chemical composition of the vocal fold ligaments.

#### 4. Arrangements against Traumatizing Forces Caused by Pulling

• A pulling force can act on the vocal fold in three directions: sagittal, vertical and medial. A vertical pull is caused by the trachea and is present in the living adult between 30 and 1480 gms, according to measurements by Zenker. A medial pull is caused by the negative pressure of the air stream (Bernoulli effect). Both these forces have been discussed in a previous paper

(Sonninen et al., 1972). In this paper we will concentrate on the sagittal (lengthwise) pull.

The vocal ligament is about  $2 \times 3$  mm in diameter in adults. It is composed by the cranial part of the conus elasticus and does not show a clear demarcation from it. The collagen fibres run mainly parallel, attaching firmly to the thyroid and arytenoid cartilages (processus vocalis). In addition this fixation is strengthened by elastic fibres so that the weakest points of the ligaments are not the fixation points. Collagen fibres have firm connections also with the epithelium and perimysium of the vocal muscle by the 'lamellar system' (Mayet, 1961). This system is horizontal in its cranial part but becomes more and more vertical in the conus elasticus. The elastic fibres form a network in the vocal ligament and the conus elasticus. In addition they form a special subepithelial network connecting the epithelium with the vocal ligament according to the principle of "venetian scissors" (Mayet, 1961).

#### Own Experiments with Human Vocal Ligaments<sup>1</sup>

We have performed earlier in this laboratory a series of elasticity tests with excised vocal folds within the range of 0–50 grams (Damste & Wieneke, 1973). A typical curve is shown in Fig. 1. In order to get a rough idea about the strength of the vocal ligaments in adult humans we also did some tests with much larger stress on excised vocal cords.

#### Method

The anterior part of the thyroid cartilage with the vocal ligaments attached to it was fixed by hanging it over a bar of diameter 3 mm and clamping it firmly. This clamp was attached to one arm of a balance. The ligament (including the cranial part of the conus elasticus) was, without muscle tissue, about  $2 \times 3$  mm in diameter. The posterior part of the vocal ligament was fixed by clamping the arytenoid cartilage.

<sup>1</sup> The experiments were carried out in the Phoniatric Department in Utrecht with the technical assistance of Mr A. C. G. van Bergen.

section of the cricothyroid muscles was 0.5 cm<sup>2</sup>. Therefore the maximal pulling force caused by the cricothyroid muscle alone may be great enough to cause microbreakages of the collagen fibres in the vocal ligaments. The probability is further increased as there is a synergetic pulling effect of the external laryngeal muscles (Sonninen, 1968). Finally, in addition to these pulling forces, there are the forces transmitted from the air stream to the vocal folds which, as was discussed previously, can also be traumatizing.

It is a tempting hypothesis that microruptures of collagen fibres may occur by injudicious use of the voice. It is evident, however, that more experiments are needed to solve the problem of vocal strain.

### ZUSAMMENFASSUNG

Die Frage nach den Ursachen von Stimmschäden bei funktioneller Überbelastung wird teilweise mit Hinweisen auf den morphologischen Bau der Stimm Lippen beantwortet. Experimentelle Belastung des Stimm Lippenligaments hat nicht eindeutig die Hypothese gestützt, dass das Brechen von Kollagenfasern eine Rolle spielen könnte.

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## RELATIVE MOVEMENTS OF THE THYROID AND CRICOID CARTILAGES ASSESSED BY NEURAL STIMULATION IN DOGS

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(Received January 1, 1974)

The literature abounds with contradictory answers to the question whether it is the cricoid or the thyroid cartilage that moves during changes in the fundamental frequency of the voice. If we assume that the cricoid is less resistant to movement than the thyroid, it is logical that it should be the moving member at the cricoid joints. This hypothesis was tested and confirmed in 15 dogs by stimulating the superior and recurrent laryngeal nerves, which supply the cricothyroid and cricoid muscles respectively. When the superior laryngeal nerve was stimulated, the cricoid arch always moved upward. It always moved backward, i.e., caudad when the recurrent nerve was stimulated. At no time did any of the thyroid cartilage result from stimulating the laryngeal nerves.

Whether it is the cricoid or the thyroid cartilage that moves as the greater propensity for movement at the cricothyroid joints is one of the most of the unanswered questions in laryngeal physiology. This is of particular interest when considering how alteration of fundamental frequency of voice is accomplished. The existence of a positive correlation between vocal frequency and vocal fold length is well established. This relationship can be defined by the distance between the cricoid fold attachments on the arytenoid and thyroid cartilages. Since the arytenoids are attached to the cricoid cartilage, the position of the cricoid relative to the thyroid cartilage is a significant determining factor of vocal pitch.

From Fig. 1A it can be deduced that contraction of the cricothyroid muscles will influence this

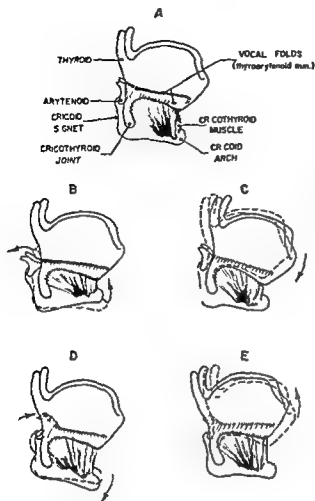
spatial relationship, and consequently the length of the vocal folds.

Ambiguity about which of these two cartilages moves in response to cricothyroid muscle contraction can be traced back to the middle 1800's (Tschiasny, 1944). Unfortunately, the reader of contemporary literature finds that this issue still has not been resolved. The plethora of recent papers on the topic of fundamental frequency of phonation illustrates that this vocal attribute is of great interest to researchers and clinicians. In the light of this interest, a persisting ignorance of the mechanisms of pitch change is indefensible. Resolving the basic question of which cartilage moves to influence vocal fold length seems obligatory before a comprehensive understanding of pitch-changing mechanisms can be developed.

Many investigators including Hooper (1883), Tschiasny (1944), Vennard (1967, p. 54) and Broad (1973, p. 134) contended that there is a dorso-cranial rotation of the cricoid around an axis passing transversely through both cricothyroid joints. This is represented in Fig. 1B. In each of the few experimental studies known to the authors, the cricoid has been identified as the moving member of the joint. Still, many authors contend that movement of the thyroid cartilage is responsible for altering the relative position of the two cartilages.

Based upon radiographic observation of the thyroid cartilage in singers, Sonninen (1956)

This study was partially supported by grants from the American Laryngological Association and NIH NS 05658 (USPHS).



1 Salient structures and possible movements of the thyroid and cricoid cartilages. (A) Identification of pertinent structures. (B, C) possible movements due to cricothyroid muscle contraction. (D, E) possible effects resulting from thyroarytenoid muscle activity. Dashed lines represent positions at rest.

was led to believe that anterior translation of the thyroid accounted for 3 out of 4 mm of vocal fold elongation associated with changing pitch from low to high notes. His data, however, may be based upon artifact. With sufficient upward rotation of the cricoid arch, the superior aspect of the cricoid signet may approximate the rigid posterior pharyngeal wall. Continued elevation of the arch, therefore, would cause forward displacement of the caudad portion of the cricoid signet and consequently the thyroid cartilage which is attached to the cricoid. This displacement could be interpreted as translation of the thyroid when attention is focused only on that

structure. Disregarding the possibility of this fact, his results are not compatible with physiological facts. For example, if the vocal folds are changed by translation of the thyroid cartilage, the ligamentous capsules surrounding the thyroid joints and an antero-posterior motion of the articular surfaces of the joints would be prerequisite. Dissection of these joints, however, does not reveal elongated articular surfaces. Because of this, and of the fact that translatory movement is diminished by the ligaments when they are tightened by the capsules, sliding action at the joints does not seem likely.

A more popular concept of thyroid cartilage motion is exemplified by Zemlin (1968) and Foxen (1968, p. 166). They, along with other authors, speculate that a vertical movement of the anterior aspect of the thyroid cartilage is the prime mover, the folds by moving the anterior aspect away from the arytoids as pictured in Fig. 1B. Pressman (1942) also indicated that the cricoid cartilage was the prime mover, however, he contended that it moved in a dorso-ventral pattern. Tschiasny's critique (1944) of this viewpoint apparently influenced him, for he subsequently wrote "either the cricoid cartilage is tilted upward or backward or the reverse, and the thyroid cartilage is rotated upward." (Pressman & Kelemen, 1935). Later, however, two experimental studies were published which verified cricoid motion.

The cricothyroid muscles with their laryngeal nerve innervation constitute a unit which influences the position of the cartilages. The nature of this influence depends upon which cartilage is the more fixed structure. If the thyroid is relatively fixed, stimulation of the laryngeal nerves should result in rotation of the cricoid as pictured in Fig. 1B. On the other hand, if the cricoid is more stable, the thyroid would move as represented in Fig. 1C.

The effect of the thyroarytenoid muscle on the vocal folds has not been reported, but seems plausible. It is contained in the vocal folds which are

teriorly to the arytenoid cartilages and course upward and forward to the thyroid, anterior to the thyroid cartilage is relatively immobile, activity in these muscles through stimulation of recurrent laryngeal nerves should cause depression of the cricoid arch, illustrated in Fig 1. This would result from shortening of the muscles causing forward displacement of the arytenoids and anterior movement of the cricoid to which the arytenoids are attached. If the cricoid is the more stationary cartilage, the superior aspect of the thyroid would elevate as presented in Fig 1E. Confusion as to which cartilage has the greater density for movement would be clarified by varying which represents the greater mechanical impedance to motion, i.e., the more 'fixed' member of the joints. The thyroid cartilage is larger and has the greatest extrinsic muscular attachments both in number and area. It would *a priori* possess the greater impedance to movement. Therefore, it is hypothesized that rotation of the cricoid arch rather than inferior directed thyroid cartilage rotation is expected with stimulation of the superior laryngeal nerves, stimulation of the recurrent laryngeal nerves should produce cricoid arch depression rather than upward rotation of the thyroid cartilage.

## METHOD

Experimental investigation of these hypotheses in humans is not possible, therefore, use of an animal is required. Many investigators have already established the precedent for using the canine larynx as the model of choice. Our dissections show great similarity between human and canine larynges in terms of size of the cartilages and their suspensory system. Responses of the thyroid and cricoid cartilages to stimulation of both superior and both recurrent laryngeal nerves were observed in each of 10 dogs approximately 17 kg in weight. Deep anesthesia of each animal was obtained using a mixture of Innoval<sup>1</sup> followed by 4-5 cc of Sodium

<sup>1</sup> Lemark of Pitman-Moore Inc., Washington Cross, New Jersey 08560

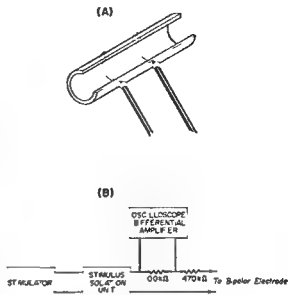


Fig 2 Instrumentation (A) Bipolar stimulating electrode (B) block diagram of stimulating equipment

Pentobarbital (50 mg/ml) with supplemental injections of the latter to maintain a deep anesthesia level. With animals in anatomical position, a midline incision was made from below the hyoid bone to near the sternum. Both right and left recurrent and external branches of the superior laryngeal nerves were dissected. These were elevated to permit attachment of bipolar stimulating electrodes. In early experiments an elevated nerve was placed onto wire hook electrodes. Because of tissue injury, use of these was discontinued and a different type of electrode was employed (see Fig 2A). The unique construction of these electrodes deserves elaboration to provide relevant details of instrumentation for replicability purposes. Two small diameter (0.28 mm) nichrome wires were passed through and partially around the inside circumference of Silastic<sup>®</sup> Medical Grade Tubing (0.78 inch inner diam) keeping the inter-electrode distance at 3 mm. A narrow section along the length of the tube was removed so that a nerve could be laid on the electrode wires which are perpendicular to the path of the nerve. To prevent continued stretching and drying of nerve tissue, the electrode assemblies and nerves were coated with diluted Silastic<sup>®</sup> 382 Medical Grade Elastomer and

buried in the neck. The lead off wires were insulated by feeding them through Silastic® Medical Grade Tubing (0.21 inch inner diam.)

Constant current monophasic stimuli were obtained from a 570 kΩ resistance in series with the output of a stimulus isolation unit (Grass SIU no 4 or SIU no 467B). A rectangular waveform stimulator (Grass S4 or S8) provided pulses of 1 msec duration at 100 Hz. Current intensities ranged from 100 to 200 μA and could be verified by use of a high impedance (10 MΩ) differential input oscilloscope. This instrumentation is represented in Fig 2B.

Identification of the nerves during dissection was verified by viewing the effects of neural stimulation on the glottis. Superior laryngeal nerve stimulation was confirmed by an elongation of the vocal folds and glottal deviation posteriorly to the opposite side of stimulation. Selection of recurrent laryngeal nerves was verified by adduction of the vocal fold on the side of stimulation or asymmetric bilateral adduction which usually was stronger on the stimulated side. Orders of nerve dissection and experimental stimulation followed no prearranged plan. Responses to neural stimulation were repeatedly obtained in each animal over a 2 to 5 hour period with intervals ranging from 10 to 30 min.

## RESULTS

The effect on cricoid and thyroid cartilage movements from stimulation of each superior and each recurrent laryngeal nerve was observed by noting the position of these structures without stimulation relative to an external laryngeal landmark and then with stimulation. The most dramatic aspect of the results was the consistency of cricoid movement in response to stimulation of the two sets of nerves. Specifically, when each of the paired superior laryngeal nerves was stimulated the anterior arch of the cricoid cartilage always moved in a cephalad direction while the thyroid cartilage in every one of the 15 dogs remained immobile. Stimulation of the recurrent laryngeal nerves also produced cricoid motion. Without exception this was a caudad

movement of the cricoid arch. The cartilage position always appeared to be unaffected by induced recurrent nerve stimulation.

Position of the head as a potential mechanism of the thyroid cartilage (by way of the strap muscles attaching to the larynx) was investigated in one animal. The experiment previously described was repeated with the neck extended and then with the neck flexed. No differences in responses of the cartilages were observed between the two conditions. Thus, head position does not appear to be an independent variable influencing the results of this study.

Demonstration that the results of this study are not specific to dogs would enhance the extrapolation of their implications to human laryngology. Therefore the experiment was repeated on a *Macaca nemestrina* monkey. Stimulation of the superior laryngeal nerves affected the position of the thyroid cartilage in a manner similar to that in the dog. Recurrent nerve activity also caused the position of this cartilage to change. Position of the cricoid cartilage was unaffected by neural stimulation. Results of this study, therefore, are restricted to dogs and can be induced in other species as well.

## DISCUSSION

Conformity of the data permits unequivocal acceptance of the two experimental hypotheses which contended that the cricoid is the anterior member of the two cartilages contributing to the cricothyroid joints. Thus it may be concluded that the thyroid cartilage system has a greater relative mechanical impedance to movement than the cricoid system. This contention seems more credible if the tonus in the strap muscles attaching to the thyroid cartilage were considered as expected in an unanesthetized condition. The study supports the contention advanced by Hooper (1883), Tschiasny (1944) and others (1967, p. 54) that the cricoid is the posterior member of the joint. Results of this study conflict with the speculation perpetuated by Lerman (1942, 1955), Zemlin (1968, p. 111) and others (1959, p. 321, 323), Hall & Coleman (1967, p. 111).

and Foxen (1968, p. 166) that the thyroid (or both cartilages) rotates in response to thyrohyoid muscle contraction. It should be pointed out that a sliding action between the thyroid and cricoid cartilages was never observed. Thus, the proposition of Sonninen (1956) and Lemlin (1968, p. 157) that forward advancement of the thyroid cartilage appreciably accounts for vocal fold elongation is not supported experimentally by our results.

What extent changes in the position of the laryngeal subjects might influence the results of this study could not be investigated. Possibly changes in orientation (for example, from an anal position to standing on all four legs) might have significantly altered the gravitational vectors and elastic restoring forces on the laryngeal systems so that their relative mechanical impedance to motion would have been altered. However, the lack of existing data on these variables and their interactions obstructs speculation as to their importance to this study. A change in orientation as a possible factor influencing the physiology of the larynx would seem to be a subject-matter for future investigation.

Differences in results between this experimental investigation of function in dogs and descriptive studies of human behavior may be partially explained by 'training effects'. Sonninen (1956) found that professional singers producing increased pitch levels generally moved the larynx upward and forward (presumably by involvement of the extrinsic laryngeal muscles). He also showed dorso-cranial rotation of the thyroid and concomitant ventro-caudal rotation of the cricoid cartilage. Not all ten of his singers, however, demonstrated similar cricoid movement; four subjects seemed not to rotate the cricoid and one produced rotation in the opposite direction. The lack of uniformity of his data might reflect differences in training among subjects. By contrast the present study negates training effects and yields different but homogeneous results. It would be interesting to determine the extent to which the upper limits of cricoid range could be developed by individuals producing high cricoid rotation without involvement of

extrinsic laryngeal muscle activity. According to Vennard (1967, p. 109) and implications drawn from Greene (1964, pp. 119-142), this method of producing increments of vocal pitch would seem to be more desirable than behavior exhibited by Sonninen's subjects (*op cit*).

## ACKNOWLEDGEMENTS

Dr M. H. Newman, Assistant Professor in the Department of Otorhinolaryngology, University of Michigan Medical School, initially performed the surgical procedures and trained the authors in this technique. He also was instrumental in developing our awareness of thyroarytenoid muscle contraction as a potential force influencing position of the cricoid arch. Mr Ambrosio Rodriguez played a significant role in developing the author's surgical skills and in teaching animal care. Great indebtedness is felt for the participation of these men.

## ZUSAMMENFASSUNG

Man findet in der Literatur viele sich widersprechende Antworten auf die Frage, ob der Krikoid oder der Thyroideknorpel sich bewegt, wenn die Grundfrequenz der Stimme sich verändert. Nimmt man an, dass der Krikoid sich leichter bewegen lässt als der Thyroid, so wäre der Krikoid das sich bewegende Glied am Krikothyroid-Gelenk. Diese Hypothese wurde geprüft und durch Experimente an 15 Hunden bestätigt, in denen die NN. laryng. superior und recurrens stimuliert wurden, die den M. cricothyroideus bzw. thyroarytenoideus innervieren. Bei Reizung des N. laryng. superior bewegte sich der Krikoidbogen immer nach vorne. Bei Reizung des N. recurrens bewegte sich der Bogen immer in kaudaler Richtung. In keinem Fall bewegte sich nach der Stimulierung dieser Nerven der Thyroidknorpel.

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## SWALLOWING DYSFUNCTION IN THE BRAIN-DAMAGED WITH DROOLING

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**Abstract** The act of swallowing in nineteen brain-damaged patients with severe drooling has been studied by means of physical examination and intraluminal pressure recordings of the pharynx and oesophagus. The study was controlled by X-ray. The study formed part of an examination preparatory to surgery designed to reduce severe drooling. All patients had swallowing disorders. The most usual pathological findings were un-coordinated tongue movements, delaying of the bolus in the mouth and pharynx, high tone and spastic contraction of the pharyngo-oesophageal sphincter and dys-coordination between the pharynx and sphincter. The disorders of the oral and pharyngeal phases of swallowing seemed to be causative factors of drooling.

The act of swallowing comprises a complex series of symmetrical excitations and inhibitions of the muscles of the mouth, pharynx and oesophagus. The sequence is supposed to be coordinated by a swallowing centre of which has been discovered in the reticular substance of the medulla oblongata (Doty

1967). The act of swallowing is usually studied by X-ray and intraluminal pressure recordings. It is generally divided according to Sandstedt (1817) into three stages: the oral, pharyngeal and oesophageal stages. Though limited to the pharynx and oesophagus, Mansson (1967) provides a good description of their movements in deglutition.

Disturbances in the mechanism of swallowing can have a central nervous cause, usually a brain lesion resulting in paralysis of the pharynx and larynx (Sjoberg 1939, Sandberg & Thiseus 1967). Other neurological

causes are extra pyramidal diseases such as athetoid forms of cerebral palsy, parkinsonism and tardive dyskinesia after taking phenothiazines (Massengill & Nashold 1969). The latter is connected with choreo-athetoid movements and difficulty in swallowing and talking caused by bucco-lingual movements. Children suffering from cerebral palsy with athetosis can present difficulties in swallowing sometimes resulting in recurrent bronchial aspiration and pneumonia (Matsaniotis et al. 1967).

Drooling is a considerable handicap amongst individuals with cerebral palsy who have passed infancy. Enfors & Lundberg (1968) estimate that about 10% of them are droolers. Cine radiographic examinations of the swallowing in children with cerebral palsy and drooling have shown pathological tongue movements but normal pharyngeal and oesophageal deglutition phases (Wilkie 1967).

### *Purpose of the present investigation*

Drooling might be due to hypersalivation and/or an insufficient mechanism for the removal of saliva. The present investigation is an analysis of swallowing in a group of brain-damaged individuals with severe drooling to find out if their drooling is caused by disturbances in one or more of the swallowing stages. This is of particular interest since the patients can be sufficiently fed. The examination formed part of a study preparatory to surgery in order to reduce severe drooling.

Table I

Type of cerebral palsy syndrome	No of patients
Spastic hemiplegia	1
Spastic diplegia	3
Spastic tetraplegia	10
Athetosis	1

## MATERIAL AND METHODS

The series comprises 19 persons between 7 and 46 years old (10 males, 9 females) with brain damage and severe drooling, all except 2 being cared for in an institution for mentally retarded. Fifteen patients have cerebral palsy and the distribution between different types according to Ingram (1964) is seen in Table I. Epilepsy in the form of grand mal is seen in 11 patients among whom 5 also have astatic myoclonus epilepsy. Administered drugs that could possibly influence salivation and/or swallowing are listed in Table II.

### Physical examination

The tongue, palate and pharynx were examined in order to detect any paresis. The behaviour during the oro pharyngeal phases of the swallowing was observed, special attention was paid to mouth, tongue and jaw movements and the timing of coughing.

### Intraluminal pressure recording

For determination of the intraluminal pressures of the pharynx and oesophagus a standardized technique was employed mainly according to the principles of Code et al (1958). The technique has been used for several years in our department. Pressures were recorded by means of three polyethylene catheters fastened together (Intramedic PE 100 ext diam 1.5 mm). A 4 mm lateral opening was made in each catheter, which was plugged just distally to the opening. The openings were located 5 cm apart. The catheters were waterfilled and flushed intermittently during the examination. They were connected to 3 pressure transducers EMT 34 (Elema Schonander, Stockholm) at

estimated oesophageal level and a transducer was connected to an electromagnet EMT 31. In a few restless cases the signal had to be filtered to reduce disturbance in the registration. A Mingograf 81 film and fluid jet recorder registered the pressures at a paper speed of 10 or 50 mm/sec. The apparatus was calibrated to atmospheric pressure and to standardized pressures, before and after every examination.

The patient's regular nurse assisted the examination. A catheter assembly was inserted nasogastrically into the fasting patient in the supine position. Sweet juice was given and the patient swallowed it. The elevation of the larynx was observed and marked as the start of swallowing. The catheter assembly was withdrawn at 1 cm intervals through the oesophagus. At each step the pressures were registered and at deglutition. When the middle opening of the catheter assembly was at the level of the maximum resting pressure of the pharyngo-oesophageal sphincter the paper speed was changed from 10 to 50 mm/sec and pressure responses to several swallows were recorded. The higher speed of the recording paper allows detailed analysis of swallowing pressure events in the pharynx (Månsson & Sandberg, 1973a).

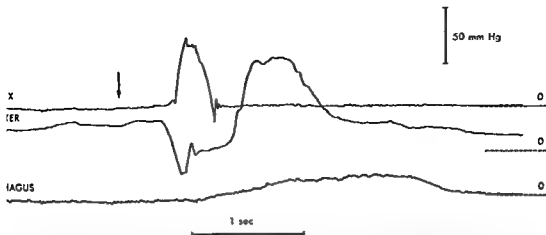
### X-ray<sup>1</sup>

During barium swallowing serial X-rays of the mouth, pharynx and oesophagus were taken at a speed of 2 pictures per second with a 70 mm Philips Odelka Camera. The

<sup>1</sup> A Ferber MBI Roentgen Diagnostica AB has performed the X-ray examination and assisted at the evaluation of the results.

Table II

Drugs	No. of patients
Benzodiazepines	7
Other skeletal muscle relaxants	1
Anticholinergics	2



Swallowing pressures at the pharyngo-oesophagus junction. Healthy female, 20 years old. The measuring points are located 5 cm apart; middle one is situated at the level of maximum pressure of the pharyngo-oesophageal or. The dotted lines represent atmospheric pressure.

sure levels. The arrow indicates the start of swallowing. The sphincter is relaxed during the whole pharyngeal contraction thus allowing draught to pass. Then draught is pushed onwards by the sphincteric contraction followed by the oesophageal peristalsis.

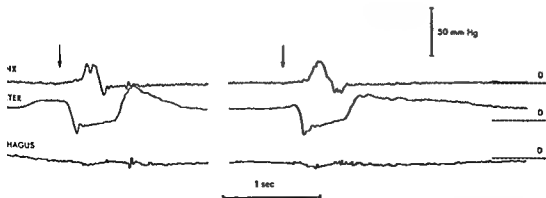
examined with frontal and right or left or 45° oblique projection in the same position as at the pressure recording, i.e. the position. Thick barium contrast was used after dilution so that it could be given through a straw.

## RESULTS

### Visual examination

Analysis of tongue, palate, or pharynx was made in any of the 19 patients. They swal-

lowed willingly the juice and barium given to them but in most cases with obvious difficulty. They showed pronounced uncoordinated tongue movements in order to swallow and seemed to have difficulty in placing the draught in a suitable position for swallowing. Two cases obviously held the draught in the mouth and pharynx for a prolonged time before they finally swallowed. All patients except one swallowed the draught without coughing (see Fig 7).



Spastic tetraplegia. 19-year-old female. The catheter positions as in Fig 1. The contractions of the pharynx and the sphincter are weak, that of

the oesophagus undetectable. The sphincter relaxes to sub-atmospheric level and is relaxing during the whole pharyngeal contraction.

Table III Pathological findings at intraluminal pressure recordings of the pharyngo oesophageal sphincter 19 patients

	No of patients
<i>Resting pressure</i>	
High (>45 mm Hg)	9
Low (<10 mm Hg)	2
<i>Relaxation</i>	
Poor	4
<i>Contraction</i>	
Spastic	8
High amplitude	1
Weak	1

### Intraluminal pressure recording

Fig 1 shows the swallowing pressure curves from a healthy volunteer

A characteristic feature of the registrations of the present investigation was the great variation within the same brain damaged individual from one swallowing to the next, in the pharynx pharyngo oesophageal sphincter, as well as in the oesophagus. None of the manometrical examinations was regarded as normal.

The pharyngeal contraction was essentially normal in all cases except 3: the one had a raised amplitude (90 mm Hg), the second had polyphasic course and the third was intermit-

tently weak (Fig 2). The type and frequency of pathological findings in the pharyngo oesophageal sphincter are shown in Table III. The most common disturbances were high and spastic contraction, i.e. contractions of high amplitude, long duration and a plateauing course (Figs 3 and 4). Of the 19 patients with high sphincter tonus 6 also had high amplitude in the contraction of the pharyngo-sphincteric dys-coordinated sphincteric contraction starting before the pharyngeal contraction had ended occurred in 7 of whom had a spastic contraction (Figs 4 and 5). In one other case it was observed that the contraction of the oesophagus was consistently premature in relation to the contraction of the sphincter.

In the oesophagus the peristalsis was present in 10 of the 19 patients. Motor disturbances were weak segmental contractions in the distal third of the oesophagus corresponding to the smooth muscle part, were registered in 5 patients, none of them receiving anticholinergic drugs. 4 of these 5 had normal peristalsis in the oral third of the oesophagus. In 4 cases disturbances in the motility of the oesophagus were seen with general hypomotility or contractions between peristaltic and segmental contractions.

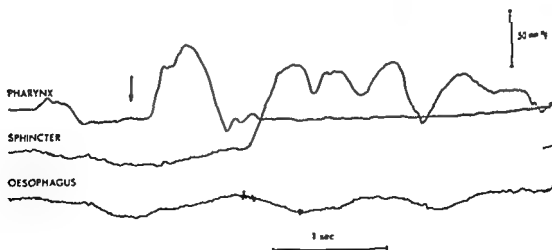
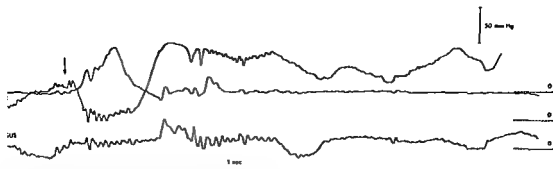


Fig 3 Athetosis 10-year-old female. The same catheter positions as in Fig 1. The sphincteric contraction has a long and undulating course. The pres-

sure variations in the oesophagus represent oesophageal contractions.



Spastic diplegia. 11 year-old female. The same positions as in Fig. 1. The sphincter contracts fully. There is a slight dys-coordination between

the pharynx and the sphincter. The premature oesophageal contraction has a long irregular course.

cases a delaying of the barium bolus in mouth at isthmus faucium or in the pharynx as noted (Fig. 6). Hypopharyngeal diverticulum did not appear in any case. In one bronchial aspiration was observed (Fig. 5) (also Fig. 5).

#### Comparison between manometry and X-ray

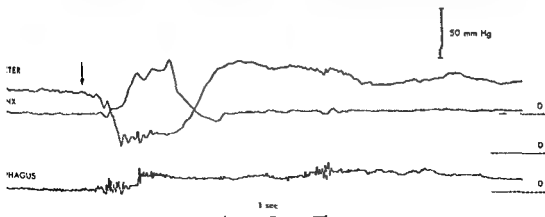
In 3 cases, where the contrast was delayed at hypopharynx, manometry showed pharyngo-sphincteric dys-coordination. 2 patients had long pharyngo-oesophageal sphincter at X-ray examination and they had high sphincter tonus at manometry. One patient with spastic diplegia showed no sphincter tonus

at manometry and the pharyngo-oesophageal sphincter could not be detected on X-ray (Fig. 8). However, in swallowing the sphincter's other manometric characteristics were present.

In all 19 cases the manometric examinations thus showed motility disorders, which in 2 cases were only slight. In one of these latter cases, however, X-ray showed a pathological finding with a pronounced delaying of the barium at isthmus faucium.

## DISCUSSION

The present investigation shows swallowing disorders in brain-damaged persons with drooling at physical examination, intraluminal pres-



Spastic tetraplegia. 26-year-old male. The same positions as in Fig. 1. The sphincter is hyaline. It does not relax to atmospheric pressure and

starts to contract while pharyngeal contraction still proceeds. No oesophageal contraction is recorded.



a



b



c



d

recordings and X ray. Impaired removal of saliva with drooling due to dysfunction in larynx-oesophagus was earlier observed on patients without central nervous disease but in peripheral nerve injury (Sandberg 1968) they showed increased tonus of the laryngo-oesophageal sphincter and hypomotility in the oesophagus after total gastrectomy and vagotomy.

The material in our study was homogeneous regarding the presence of brain damage and drooling but heterogeneous regarding the degree of brain-damage.

The diazepam derivative nitrazepam administered to patients with minor motor epilepsy was reported to give hypersalivation and impaired breathing which might be due to hypersecretion of salivary and bronchial glands (Sandberg 1968). The symptoms mentioned might also be due to oral saliva stagnation and tracheal aspiration. In our study however no special pathologic pattern in swallowing was detected in the 7 patients receiving benzodiazepines. Further work is necessary to investigate the effects of such drugs on swallow-

ing. The X ray examinations showed the morphology of the pharynx and oesophagus but gave only limited information about the motility. The majority of the patients were because of their mental state not capable of cooperating in simultaneous cine radiographic examination and intraluminal pressure recordings. Such an investigation would have given further information of the motor events of swallow-

ing. On X ray the delaying of the barium contrast in the mouth at the isthmus faucium and in the pharynx which was registered in all patients agrees well with the difficulty of slowing fluids. Stopping of the bolus at the isthmus was earlier observed in mentally



Fig 7 Spastic tetraplegia same patient as in Fig 5. Swallowing study showing barium retained in the pyriform sinuses and tracheal aspiration.

retarded patients without drooling (Massengill 1968) and was also found in patients with pharyngeal paralysis (Kuchel et al. 1969). However in the present study physical examination showed no pharyngeal paralysis.

46 Spastic tetraplegia. A 46-year old female. Swallowing study performed at a speed of 2 exposures per second. The bolus is delayed in the mouth and pharynx for 2 seconds without being transported into the pharynx.





Fig. 8 Spastic diplegia 14-year-old male. Swallowing act with barium in the mouth, pharynx and upper oesophagus. The pharyngo-oesophageal sphincter is not visible.

Disturbances in the oral swallowing phase seem to be an important causative factor for drooling: our patients showed obvious oral difficulties with un-coordinated tongue movements in swallowing. Children with cerebral palsy and drooling have been observed by cine radiography to have swallowing disorders of the oral phase (Wilkie 1967). Retropositioning of the ducts of the great salivary glands is a surgical method of overcoming the functional oral obstacle by passing saliva to the pharynx.

In cases where the pharyngo-oesophageal sphincter relaxation is sufficient this operation reduces drooling significantly.

Weak pharyngeal contraction or incomplete sphincteric relaxation could also result in motor dysfunction at the oesophageal junction. Practically this is exemplified by the patient with a high resting tone at swallowing: he had a high resting tone and an insufficient relaxation (Fig. 1 and 7). The other 8 patients with a lower resting tone had sufficient relaxation during swallowing and they did not aspirate. A good relaxation probably also saved a patient from aspiration though she was obviously in her swallowing and had a weak pharyngeal contraction (Fig. 2).

A possible reason why the swallowing of food goes so well in spite of no drooling registered might be the absence of changes in sensitivity. In experiments on volunteers, surface anaesthesia of the base of the tongue and pharynx has been reported to cause swallowing disturbances, coughing and dys-coordination at the pharynx, which illustrates the significance of sensitivity (Månsson & Sandberg 1973).

Measures against drooling which result in a strong reduction of the amount of saliva secreted seem to be a disadvantageful operation. Because of difficulties in swallowing, patients are probably dependent on a constant supply of saliva, since this provides a stimulus against the reflexogenic activity in the mouth and pharynx in order to start the swallowing reflex.

Drooling of brain-damaged persons can be treated by retropositioning of the sublingual ducts combined with ligation of the sublingual ducts in order to overcome the hindrance to the saliva transport (Månsson 1973). The present investigation of 10 patients has shown that swallowing disorders at the pharyngo-sphincteric junction are common and are of probable causal importance in drooling. In cases where drooling is sufficiently reduced by the retropositioning

a sphincterotomy might be the logical  
 py for those who have a severe disorder  
 ie pharyngo-sphincteric function

## ZUSAMMENFASSUNG

Schlindbewegung von neunzehn Personen mit Ge-  
 chaden und starkem Geifer sind mittelst physi-  
 her Untersuchung und intraluminaler Druck-  
 ung in Pharynx und Ösophagus studiert worden.  
 Morphologie wurde mit Röntgen kontrolliert. Die  
 rsuchungen gehörten zu den Kontrollen die vor  
 Operation mit dem Ziel einer Verminderung des  
 rs gemacht wurden. Alle Patienten zeigten  
 cktstörungen. Die gewöhnlichsten pathologischen  
 eckungen waren unkoordinierte Bewegungen der  
 e Verlangsamung des Bolustransportes in Mund  
 Pharynx hoher Tonus und spastische Kontrak-  
 les pharyngoösophagealen Sphinkters und schliess-  
 mangelnde Koordination zwischen Pharynx und  
 öster. Die pathologischen Befunde der oralen und  
 ngealen Phase der Schlindbewegung scheinen von  
 ilder Bedeutung für den Geifer zu sein.

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## EXPERIENCE WITH REINKE'S OEDEMA

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**Abstract** In the course of their 5 year period of study the authors have observed not only the classic picture of Reinke's oedema but some of its modifications too by applying microlaryngoscopy. The most frequent of these modifications was the phenomenon of oedematous protrusions on the border of the anterior and the median third of both vocal cords, on their free edge and on part of the inferior surface. The histological analysis of these changes revealed the same finding as that in classic Reinke's oedema, i.e. accumulation of fluid in the loose subepithelial tissue. The authors consider the mechanical factor, i.e. misuse and overuse of the voice, responsible for the development of such circumscribed oedemas on the vocal cords. The authors were able to corroborate the known facts about the strictly defined borders of Reinke's space by experimental work on corpses. All patients were treated by means of microlaryngosurgery under general endotracheal anaesthesia. No recurrence was seen following this therapy.

The well known picture of Reinke's oedema, described as early as 1891 and induced experimentally by Hajek, has been encountered fairly often in our practice too. Hajek succeeded, by means of subepithelial injections of methylene blue into the region of the vocal cords, in causing experimentally an oedema localized to the vocal cords only—just as in Reinke's oedema—and established subsequently that the fluid remained strictly confined to the region of the loose subepithelial tissue of the vocal cords.

Reinke (1897) found an anatomical justification for such accumulation of fluid in the subepithelial space of the vocal cords. He established that the subepithelial space of the vocal cords, consisting of scattered connective fibrils, was confined on all sides by dense connective tissue which prevented further penetration of the fluid.

These border lines of Reinke's space: on the cranio-caudal side, the linea arcuata and inferior, the sites where the squamous epithelium of the vocal cords turned into the oral epithelium. The anterior border of Reinke's space consists of an elastic membrane in the region of the anterior commissure where the connective tissue of Reinke's space ends and the connective tissue begins.

The posterior border is the site where the arytenoid process of the vocal cords firmly attaches to the vocal process of the arytenoid. On histological specimens, Mayet (1961) found scattered connective fibrils in Reinke's space arranged in lamellar order and that this lamellar structure makes possible the development of the oedema as the result of a certain type of irritation.

The first description of Reinke's oedema, given by Hajek, is still accepted today. The vocal cords show a spindle shaped thickening covered by thin epithelium through which a fluid shines. Hajek compared this picture with that of nasal polyps.

No uniform opinion has as yet been reached concerning the etiology of Reinke's oedema. One group of authors, represented by Reinke (1897) and Mann (1958), maintains that Reinke's oedema is the result of chronic inflammation of the upper parts of the respiratory tract, i.e. of the paranasal sinuses and that, due to permanent irritation, leads to the development of the oedema. Kleinsasser (1968), on the other hand, emphasises that a diffuse non-



*Fig 1* Microlaryngoscopic picture of circumscribed Reinke's oedema located on the free edge and on the part of lower surface of both vocal cords

inflammatory oedema of obscure etiology involved

the contradictory opinions with regard to the pathogenesis of Reinke's oedema and our observations during microlaryngoscopy prompted us to study this problem in detail

### CASE REVIEW

In the last 5 years 60 patients were examined means of microlaryngoscopy and treated for Reinke's oedema in our Department. Thus not only the classic Reinke's oedema but also a number of modifications were seen and studied. The reason why these patients reported for examination was more or less marked hoarseness which did not respond to conservative therapy. Apart from hoarseness there were marked respiratory troubles in 22 patients.

We found the picture of classic Reinke's oedema with diffuse spindle-shaped oedema of the vocal cords along their entire length and unchanged epithelium with fluid shining through in 15 of our patients. In 2 of our patients with respiratory symptoms the oedema of the vocal cords was marked to such an extent

that most of the rima was closed. In all other patients we detected by microlaryngoscopy the presence of smaller, circumscribed swellings on the vocal cords and these were of the same morphologic appearance as the classic oedema.

In most of our cases (30) these protrusions were localized on the free edge of the vocal cord and on part of its lower surface (Fig 1). However, some of these localized oedematous protrusions were seen on the upper surface of the vocal cords too. These circumscribed oedemas were most frequently found on the border of the anterior and the median third of both vocal cord. We also found unilateral oedemas which involved the entire vocal cord or only a smaller part of it, but it was seen in a few cases only.

In none of our cases were there any signs of inflammation of the vocal cords, nor of inflammatory changes in the surrounding mucosa of the larynx and trachea. In order to corroborate the statements of these authors who maintained that Reinke's oedema was the result of chronic inflammatory irritation from the upper respiratory tract, we examined the nose and the paranasal sinuses of our patients. The X-rays showed slight inflammation of the paranasal sinuses in

## EXPERIENCE WITH REINKE'S OEDEMA

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**Abstract** In the course of their 5 year period of study the authors have observed not only the classic picture of Reinke's oedema but some of its modifications too, by applying microlaryngoscopy. The most frequent of these modifications was the phenomenon of oedematous protrusions on the border of the anterior and the median third of both vocal cords, on their free edge, and on part of the inferior surface. The histological analysis of these changes revealed the same finding as that in classic Reinke's oedema, i.e. accumulation of fluid in the loose subepithelial tissue. The authors consider the mechanical factor, i.e. misuse and overuse of the voice, responsible for the development of such circumscribed oedemas on the vocal cords. The authors were able to corroborate the known facts about the strictly defined borders of Reinke's space by experimental work on corpses. All patients were treated by means of microlaryngosurgery under general endotracheal anaesthesia. No recurrence was seen following this therapy.

These border lines of Reinke's space: cranio-caudal side, the linea arca, and inferior, the sites where the squamous epithelium of the vocal cords turned to the oral epithelium. The anterior border consists of an elastic membrane in the anterior commissura where the connective tissue of Reinke's space and connective tissue begins.

The posterior border is the site where the arytenoid process of the vocal cords firmly attaches to the vocal process of the arytenoid. On histological specimens, Mayer (1961) found connective fibrils in Reinke's space ranged in lamellar order and that lamellar structure makes possible the development of the oedema as the result of type of irritation.

The first description of Reinke's oedema given by Hajek, is still accepted. The vocal cords show a spindle-shaped oedema covered by thin epithelium through which fluid shines. Hajek compared this with that of nasal polyps.

No uniform opinion has as yet been reached concerning the etiology of Reinke's oedema. One group of authors, represented by Reinke (1897) and Mann (1958), maintains that Reinke's oedema is the result of chronic inflammation of the upper parts of the respiratory tract, i.e. of the paranasal sinuses and which, due to permanent irritation, leads to the development of the oedema. Kleinsasser (1968), on the other hand, emphasises that a diffuse

The well known picture of Reinke's oedema, described as early as 1891 and induced experimentally by Hajek, has been encountered fairly often in our practice too. Hajek succeeded, by means of subepithelial injections of methylene blue into the region of the vocal cords, in causing experimentally an oedema localized to the vocal cords only—just as in Reinke's oedema—and established subsequently that the fluid remained strictly confined to the region of the loose subepithelial tissue of the vocal cords.

Reinke (1897) found an anatomical justification for such accumulation of fluid in the subepithelial space of the vocal cords. He established that the subepithelial space of the vocal cords, consisting of scattered connective fibrils, was confined on all sides by dense connective tissue which prevented further penetration of the fluid

iller oedemas it was sufficient to perform excision of the oedematous mucosa and the swelling would disappear and the vocal cord would return to normal. In more extensive oedemas the polypoid and protruded into the larynx the protruding part was excised by means of a microsurgical technique while the subglottic viscous fluid was removed by means of a pump. In these cases the vocal cord returned to normal size immediately following this operation, but the voice took several days to return. It would like to mention that in none of our cases where such a bilateral excision was performed did synechiae form between the vocal cords.

Following such interventions our patients were observed over a period from 1 to 5 years and no recurrence was seen. Whether this was due to the fact that our patients were postoperatively referred for phoniatric re-education or to the formation of a stronger connective tissue formed in the area of the incision respectively excision, preventing recurrence of the oedema in this area is, however, for time being difficult to establish with certainty.

The excised parts were histologically examined and the picture of classic Reinke's oedema was always found. The unchanged covering epithelium was raised together with the basal lamina and connective fibrils of the lamina propria were dilated by the oedema (Figs 2 and 3). Inflammatory changes were not found in any of our cases. Malignant alteration of Reinke's oedema as mentioned by some authors (Berlinger, Scheuffler, 1960) was not encountered in any of our cases.

## COMMENT

In our opinion that the main etiological factor in the development of Reinke's oedema is the mechanical factor, i.e. misuse and overuse of the voice over a prolonged period. It is evident from the anamnestic data that our patients strained their voices considerably and that 2 of them were treated for an extended period from dry cough. Four of the mechanical factors, moreover, the fact that circumscribed Reinke's oedemas

were seen most frequently precisely on the border of the anterior and the median third of the vocal cord, which is in the site of greatest friction.

We repeated the experiments which Hajek carried out in 1891 on corpses and injected physiological solution or methylene blue into the subepithelial region of the vocal cords with the aim of thoroughly inspecting once again Reinke's space and its borders and the relationship towards the epithelium and the fibroelastic membrane.

We also wished to establish by means of serial sections across individual parts of the vocal cords whether a histological factor existed to encourage the development of localized Reinke's oedema precisely on the border of the anterior and median third of the vocal cord. In the site of the *linea arcuata superior et inferior* we noticed the presence of thick connective tissue immediately below the epithelium, thus indicating that the loose connective tissue was missing.

In contrast to Mayet, we were able to trace very nicely the borderline of the *linea arcuata superior*. The anterior, the posterior and the inferior border of Reinke's space were identical in our histological preparations to those described by Reinke, Hajek and Mayet. Serial sections across individual parts of the vocal cords along their entire length as far as the *processus vocalis* revealed loose connective tissue with a few interlacing connective threads. Thus the structure of Reinke's space does not yield an explanation of the circumscribed oedema on the border of the anterior and the median third

## CONCLUSION

By means of microlaryngoscopy, in addition to the classic clinical picture of Reinke's oedema, the existence of circumscribed oedematous swellings of the vocal cords was found. Clinical and histological investigation showed that the same substrate was present in these localized oedemas as in the classic Reinke's oedema. With regard to the most frequent appearance of such oedematous swellings on the border of the

terior and the median third of the vocal cords and, under exclusion of other causes, we consider the mechanical factor to have a decisive role in the development of such an oedema. Surgical incision or excision gives excellent therapeutic results in Reinke's oedema.

### ZUSAMMENFASSUNG

Während 5 Jahren beobachteten die Autoren mittels der Mikrolaryngoskopie nicht nur das klassische Bild des Reinkeschen Ödems, sondern auch seine Modifikationen. Am häufigsten war die Erscheinung von ödematösen Schwellungen an der Grenze des vorderen und mittleren Drittels der beiden Stimmlippen, auf ihrem freien Rand und auf ihrer unteren Fläche. Die histologische Untersuchung dieser Veränderungen zeigte denselben histologischen Befund wie beim klassischen Reinkeschen Ödem, d.h. eine Flüssigkeitsakkumulation im zarten subepithelialen Bindegewebe.

Die Autoren glauben, dass für das Entstehen eines solchen zirkumskripten Ödems an den Stimmlippen der mechanische Faktor, d.h. der Missbrauch und die Überanstrengung der Stimme verantwortlich ist. Den Autoren ist es gelungen, die bekannten Tatsachen über die scharf gezogenen Grenzen des Reinkeschen Raumes durch experimentale Arbeit an Leichen zu bestätigen.

Alle Patienten wurden mittels Mikrolaryngochirurgie

in kompletter endotrachealer Anästhesie operiert. Keine Rückfälle wurden nachfolgend festgestellt.

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## STAPEDIUS REFLEX AND MONAURAL MASKING

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(Received February 6, 1974)

The masking produced by narrow band low frequency noise was measured in three subjects with total stapedius paralysis. The subjects had normal hearing up to 80 kHz. Hearing thresholds were traced using tone of fixed frequency in the range 10-100 kHz. Masking was produced by 0.5 kHz continuous noise (3 kHz bandwidth) in the intensity range 85-120 dB. Measurements were obtained both from the ear with stapedius paralysis and from the ear with normal hearing, during the acute stage of the paralysis and after recovery. Below reflex threshold, masking was equal in both ears. Above reflex threshold it was considerably greater in the ear with stapedius paralysis. The greatest difference was at 6 and 8 kHz and reached about 10 dB. After recovery masking was equal in both ears, showing that the antimasking effect of the stapedius reflex could be completely explained by its attenuation of low frequency masking noise. We conclude that the stapedius reflex has an important influence on hearing at low frequencies by decreasing the masking produced by low frequency sound.

Masking pattern of tones and narrow band noise is highly asymmetric in its frequency distribution. Sound with frequencies below the masker frequency are little affected whereas sound above the masker frequency are highly affected. At high levels of the masker the masking extends towards high frequencies. It becomes very asymmetric (Wegel & Lane, 1924, de Mare & Lane, 1950, Egan & Hake, 1950, Bilger & Durling, 1956, Ehmer, 1959a, b). It has been suggested that the stapedius reflex is of importance to limit the masking of high frequencies by low frequency sound (Stevens & Davis, 1938, Liden, 1960, Liden et al., 1964, Borg, 1972). The above-mentioned asymmetry of the masking

pattern and the efficient attenuation of low frequency sound provided by the stapedius contraction (Borg, 1968) support this hypothesis. No results of direct measurements are available that can prove that the stapedius reflex has an antimasking effect.

The aim of the present work was to determine in what way the acoustic stapedius reflex contributes to the monaural masking pattern of low frequency noise. Measurements were performed in subjects with temporary unilateral paralysis of the stapedius muscle (Bell's palsy cases). The results obtained indicate that the acoustic middle ear reflex effectively reduces the masking of high frequency sound by low frequency sound.

## MATERIAL AND METHODS

Experiments were performed on a total of 52 subjects, out of these 32 were a control group selected among university students to determine some normal features of the stapedius reflex, 20 were subjects with unilateral peripheral facial palsy (Bell's palsy). On three of these, masking measurements were performed and on 19 the attenuation provided by the stapedius reflex was determined. None of the 52 subjects was known to have had any previous neurological disease or ear disorder. All had normal ear drums and hearing thresholds within 15 dB according to ISO Standard (1964) between 0.125 and 30 kHz with Békésy audiometry.

The 32 subjects of the control group were analysed in order to establish criteria for bilateral symmetry of stapedius reflex and to determine the normal reflex thresholds with the method

This study was supported by Grants from the Swedish Research Council, the Medical Faculty, University of Umeå, the Frénckners fond, the Fromms fond and the Karolinska Institutets fond.



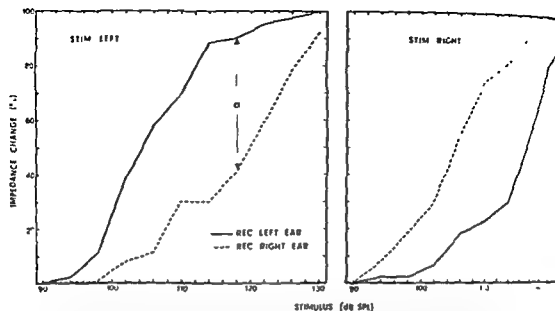


Fig 1 Stimulus-response curves of the ipsilateral and contralateral stapedius reflex of a subject in the control group measured as impedance change upon stimulation with 0.5 kHz pure tone. Amplitudes of recordings in left (—) and right (----) ear are given as a percentage of maximal obtained response in each ear. The difference

in response amplitude ( $a$ ) was measured at 4 values in the range where the response amplitude was between 10 and 100%. The average difference between the left graph and  $A'$  of the right graph was used as symmetry index  $S$  in this case = 5.2.

used. The normal variations in sensitivity of the stapedius reflex for left and right ear were determined with stimulation with 0.5 kHz pure

The difference between the response amplitudes of the ipsilateral and contralateral reflexes measured for stimulation in both the left and right ear (Møller, 1961). The distance in ordinate direction (e.g.  $a$  of Fig 1) between each pair of the stimulus response curves was determined with 4 dB interval in a large range of sound intensities. The average value of the distance was used as a measure of the difference in sensitivity between ipsilateral and contralateral reflex. Thus, two average values were obtained for each subject, one ( $A$ ) for the difference left-right and one ( $A'$ ) for the difference right-left. If the reflexes are completely symmetric the difference between the two values, "the symmetry index",  $S = |A - A'|$ , should be zero. In this control material the median symmetry index was 10 and 90% of the subjects had symmetry index below 30. The average ipsilateral reflex threshold for 0.5 kHz pure tone stimulation was 97 dB SPL (standard deviation,  $SD =$

5.0) and the average contralateral reflex threshold 102 dB SPL ( $SD = 6.4$ ).

The 20 Bell's palsy cases were selected on basis of the following criteria:

- (1) Complete loss of stapedius reflex on the same side as the facial palsy (as shown upon contralateral stimulation with pure tones up to 130 dB SPL as shown by impedance measurements).
- (2) Ipsilateral reflex threshold in the non-affected ear within  $\pm 2 SD$  of the normal average value 97 dB SPL.

A symmetry index less than 30 was used as the criterion for recovery from muscle paralysis. Nineteen of the 20 subjects fulfilled this criterion and could be evaluated of the attenuation produced by the stapedius reflex at 0.5 kHz as described by (1968). It was measured as the difference between the corresponding contralateral stimulus-response curves obtained after recovery and during paralysis.

The three Bell's palsy subjects who

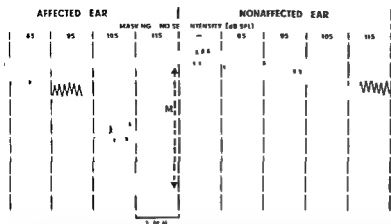


Fig. 1. Hearing thresholds of one subject with fixed Bekesy audiometry at 6.0 kHz without masking. 10.5 kHz monaural (contralateral) masking noise levels. Left side: Ear with stapedius paralysis.

(affected ear) Right side: Ear with normal stapedius reflex (non affected ear). Masking is defined as rise in threshold ( $\Delta f$ ) during presentation of the masking noise.

measurements were also lacking stapedius reflex upon ipsilateral 0.5 kHz pure tone stimulation up to 130 dB SPL. Their hearing thresholds were within 15 dB according to ISO (1964) between 0.125–8.0 kHz with audiometry. The differences in hearing thresholds between the ears were less than 10 dB. Eardrums were normal as shown by otoscopic inspection with high magnification. These subjects recovered from their stapedius muscle paralysis (symmetry index 11) and then the masking measurements were repeated.

The session was started with a determination of the masked threshold in one ear. Then a masking noise was presented in the same ear and the tracing was continued for 0.5–2 min until steady value was obtained. Measurements were performed in the affected and nonaffected ear during paralysis as well as after recovery.

The measurements were performed in a soundproof room with background noise below 30 dB SPL in the frequency range 315–8000 Hz (Bekesy & Kjaer, 1964; filter). In each session maskings were investigated at one frequency at several intensities in each ear. At least 8 hours elapsed between sessions. The hearing thresholds were determined by means of a Bekesy audiometer

(Grason-Stadler type E 800 with TDH 39 earphones) with pulsating tone (2.5 pulses per sec) of fixed frequency, slow setting. One of the subjects was investigated at the frequencies 1.0, 1.5, 2.0, 3.0 and 4.0 kHz and the two other subjects at 2.0, 3.0, 4.0, 6.0 and 8.0 kHz.

The masking noise was produced by a Bruel & Kjaer noise generator (type 1024). Bandpass filtered noise centered at 0.5 kHz and a bandwidth of 0.3 kHz (at 3 dB points) was used as the masker in all experiments. The RMS level was varied from 85 to 120 dB SPL and the various intensities were presented in a randomised fashion. The individual stapedius reflex threshold for the masking noise was determined by measuring contralateral impedance change. The ipsilateral reflex threshold for the masking noise was estimated on the basis of the subject's ipsilateral–contralateral difference for 0.5 kHz pure tone stimulation.

Calibration of the earphones used for hearing threshold determination and for presentation of the masking noise was carried out in a 6 cc coupler and a Bruel & Kjaer condenser microphone type 4132. The determination of sensitivity of the stapedius reflex was done by measurements of the sound pressure level at the eardrum by the

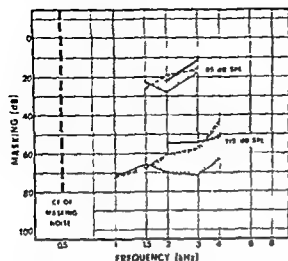
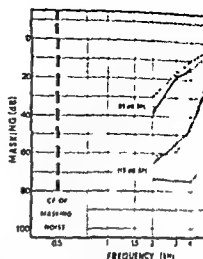


Fig. 3. Masking as a function of test frequency in 2 subjects (left and right graph respectively) at two masking noise levels (85 and 115 dB) —, affected ear during



stapedius paralysis —, the same ear after recovery of reflex — — —, non-affected ear. Dotted areas show masking during paralysis.

## RESULTS

### A. Determination of masking

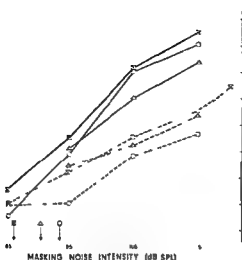
During the presentation of narrow band noise centered at 0.5 kHz, thresholds for pure tones in a wide frequency range were elevated both in ears with normal stapedius reflex and in ears with a stapedius paralysis. Fig. 2 shows a typical set of recordings of hearing thresholds obtained at 6.0 kHz without masking noise (—) and at various levels of ipsilateral 0.5 kHz noise (85–115 dB SPL). Left graph shows measurements in the ear with stapedius paralysis (affected ear) and right graph in the ear with normal stapedius reflex (non-affected ear). Though thresholds increase with increase in noise level in both ears, the magnitude of the elevation in threshold is much greater in the affected than in the non-affected ear. After the end of the noise presentation the hearing thresholds rapidly returned to the preexposure level. The magnitude of masking produced by a certain noise is defined as the difference in hearing threshold at a certain test frequency with and without masking noise (e.g. *M* of Fig. 2).

### B. Masking as a function of test frequency

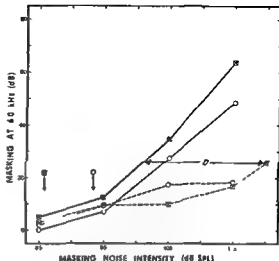
Fig. 3 shows masking produced by the 0.5 kHz noise in 2 subjects with unilateral Bell's palsy

during stapedius paralysis and after recovery of reflex. Two levels of the masking noise are used: one (85 dB SPL) below the reflex threshold and the other one (115 dB SPL) well above the reflex threshold. The thin continuous lines represent masking in the affected ear during paralysis. Heavy continuous lines show masking in the same ear after recovery of the reflex function. Broken lines indicate masking in the non-affected ear. Shaded areas show the difference in masking between the affected and non-affected ears during stapedius paralysis.

It is seen in Fig. 3 that masking decreases with intensity below reflex threshold (85 dB SPL) and is independent of function of the stapedius muscle. At high masking noise level (115 dB SPL) there is considerably more masking in the ear with paralysed stapedius muscle than in the ear with a functioning stapedius reflex. Differences (shaded areas) are further pronounced at low test frequencies and are frequency dependent. There is no difference in masking at the frequencies 1.0 and 2.0 kHz (left graph). Above 2.0 kHz there is a difference in masking that increases with frequency (right graph) and reaches maximum at 6.0 kHz (85 dB). The masking in the ear with normal reflex produced by the 115 dB noise is relatively constant at a value of 70 dB in the frequency range investigated (2.0–8.0 kHz) and is not



Masking at 40 kHz (left graph, 3 subjects) and 60 kHz (right graph, 2 subjects) as a function of the level of 0.5 kHz masking noise —, affected ear during paralysis, ----, non-affected ear. Arrows with



symbols show estimated ipsilateral stapedius reflex thresholds for the masking noise. *D* indicates shift of the curves caused by functioning stapedius reflex.

tend beyond 80 kHz. After recovery (heavy masking in the affected ear equals that in non-affected ear also at 115 dB).

The third subject the stapedius reflex did not recover within 9 months according to the above mentioned criterion. In the stage of unilateral stapedius muscle paralysis masking was measured up to 80 kHz. The results were similar to those shown in Fig. 3, right graph. This subject also showed the most pronounced difference in masking at the highest frequencies. The maximal difference was 54 dB at 80 kHz and 115 dB SPL intensity of the 0.5 kHz masking noise.

In the non-affected ear all differences between the values of the two masking measurement occasions were less than 6 dB (age 2.7 dB). This was interpreted as a good reliability of the method.

#### Masking as a function of the masker level

The magnitude of masking at 40 and 60 kHz as a function of the intensity of the masker in 3 subjects is shown in Fig. 4. The continuous lines represent masking in the affected ear during stapedius paralysis and the broken lines represent masking in the non-affected ear. The arrows indicate the

individual stapedius reflex thresholds for the masking noise estimated as described above. It is seen that masking is equal in both ears below the reflex threshold. When the masker level is raised above reflex threshold there is a rapid increase of masking in the affected ear. The corresponding increase is considerably slower in the non-affected ear. Thus reflex activity shifts the masking curve towards higher intensity and changes its slope. The shift can be measured as the distance *D* of Fig. 4 (right). This value represents the decrease in the 0.5 kHz masking noise level that is needed to obtain masking in the ear with paralysed stapedius muscle equal to that in the non-affected ear.

#### *D*: Is antimasking due only to reflex attenuation of masking noise?

Fig. 5 shows a comparison between the attenuation at 0.5 kHz provided by the normal stapedius reflex and the shift of the masking curves (represented e.g. by *D* of Fig. 4). The interrupted line is the attenuation determined as described by Borg (1968). It is based on stimulus response curves of the reflex measured as change in the ear's acoustic impedance in 19 subjects. Attenuation is 7 dB per 10 dB increase of the

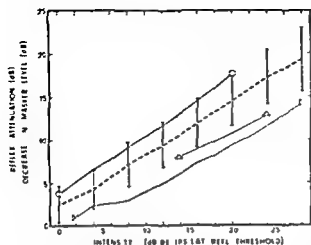


Fig. 5 Dashed line shows mean and S.D. of the attenuation provided by the stapedius reflex measured on 19 Bell's palsy subjects. Abscissa of the interrupted line stimulus intensity above ipsilateral reflex threshold for 0.5 kHz pure tone. Continuous lines show average decrease in masker level that is necessary to obtain the same masking at the frequencies 30, 40, 60 and 80 kHz in the affected ear as in the normal ear. Abscissa of continuous lines masker intensity above estimated individual stapedius reflex threshold for the 0.5 kHz masking noise. Each continuous line represents one subject. Same symbols as in Fig. 4.

stimulus intensity. This means that a raise of sound pressure in the auditory canal by 10 dB gives an increase of excitation in the cochlea by 3 dB.

The solid lines represent the average shift of masking above 20 kHz (the *D*-values) in each of the 3 subjects investigated.

It is seen from Fig. 5 that the continuous lines are close to or below the interrupted line. The good agreement between the curves points strongly to the attenuation of sound transmission as the sole explanation for the antimasking effect provided by the stapedius reflex.

## DISCUSSION

The results from the present study showed that the low frequency noise masked high frequency sounds to a much greater extent in an ear without stapedius reflex than it did in an ear with normal stapedius reflex. The decrease of masking due to stapedius activity thus improved hearing in a wide frequency range up to at least 80 kHz,

probably even to the upper frequency hearing. A normal stapedius reflex masked threshold up to about 50 dB. The masking effect can wholly be explained by the attenuation of the low frequency provided by the stapedius reflex (Fig. 5).

It might seem surprising that the frequencies below about 2.0 kHz is practically unaffected by the stapedius activity. Two reasons for this finding can be given.

(1) In the frequency range below 2 kHz the stapedius reflex attenuates both the masker and the masked sound. Thus a stapedius reflex contraction is followed by an approximate decrease in the transmission of both the masker and the masked tone through the middle ear.

(2) Since masking close to the masker frequency grows slowly as the masker level is increased (see e.g. data by Bilger & Helmer, 1959a) attenuation of the masker will affect masking less in this frequency range at greater distance from the masker.

## Functional significance of antimasking

The traditional view of the stapedius reflex is that of an attenuator of transmission of high frequency sound in the middle ear. The attenuation amounts to about 20 dB (see Fig. 5) and agrees with earlier results (Borg 1971). The finding of this study consists of the effect of hearing at high frequencies. The reflex attenuation of a low frequency masker improves hearing at high frequencies by up to 10 dB.

It has recently been shown (Borg & Zakrisson 1973) that discrimination of speech is significantly impaired in ears with stapedius paralysis at levels above the experimental threshold. The antimasking effect of the present study offers a probable explanation for this finding: the strong low frequency attenuation of speech sounds do not mask the high frequency ones in the presence of the stapedius reflex. The properties of a functional stapedius reflex and speech characteristics thus seem well adapted. The significance of the masking effect on speech discrimination has been suggested earlier on the basis of

ients who had undergone surgery for otitis media (Liden et al, 1964)

The main conclusion of the present study is that the contraction of the stapedius muscle influences the whole frequency range of hearing. This is accomplished by its selective attenuation of frequency sounds whereby their masking of frequency sounds is strongly reduced.

## ZUSAMMENFASSUNG

Drei Patienten mit einseitiger totaler peripherer Taubheit wurde die Grösse der Verdeckung im niederfrequenten Schmalbandrauschen bestimmt. Versuchspersonen hatten normales Gehör im ganzen Bereich bis 8 kHz. Die Hörschwellenbestimmungen wurden mit pulsiertem Ton bei jeweils konstanter Intensität im Bereich von 10 bis 80 kHz durchgeführt. Verdeckungsgerausch diente ein kontinuierliches Rauschen von 300 Hz Bandbreite mit einer Mittenfrequenz von 500 Hz und einer Intensität innerhalb des Bereiches von 85 bis 120 dB SPL. Die Messungen wurden durchgeführt an dem Ohr mit Stapediuslähmung wie an dem Ohr mit normalem Stapediusreflex durchgeführt und während des Stadiums der akuten Lähmung und nachdem die Stapediusfunktion wieder normal geworden war. Unterhalb der Reflexschwelle war die Grösse der Verdeckung in beiden Ohren die gleiche. Oberhalb der Reflexschwelle war sie dagegen in dem Ohr mit Stapediuslähmung durchgehend grösser. Der Unterschied war grösser für 6 und 8 kHz und konnte dort bis zu 50 dB betragen. Nach der Rückkehr der Stapediusfunktion war die Grösse der Verdeckung in beiden Ohren die gleiche. Der Effekt der Verminderung der Verdeckung durch den Stapediusreflex konnte quantitativ aus seinem Frequenzeinfluss auf das niederfrequente Verdeckungsgerausch erklärt werden. Es ist daraus zu schliessen, dass der Stapediusreflex auf Grund seiner Minderung der Verdeckung stärkere niederfrequente Frequenzanteile bewirkt. Dies hat eine wesentliche Bedeutung für das Hören von Sprachlauten im Frequenzbereich von 1 bis 8 kHz.

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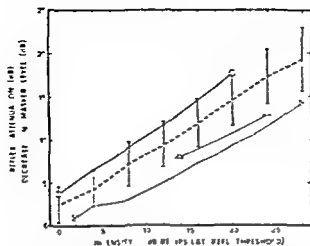


Fig. 4. Dashed line shows mean and S.D. of the attenuation provided by the stapedius reflex measured on 19 Bell's palsy subjects. Abscissa of the interrupted line stimulus intensity above ipsilateral reflex threshold for 0.5 kHz pure tone. Continuous lines show average decrease in masker level that is necessary to obtain the same masking at the frequencies 30, 40, 60 and 80 kHz in the affected ear as in the normal ear. Abscissa of continuous lines masker intensity above estimated individual stapedius reflex threshold for the 0.5 kHz masking noise. Each continuous line represents one subject. Same symbols as in Fig. 4.

stimulus intensity. This means that a raise of sound pressure in the auditory canal by 10 dB gives an increase of excitation in the cochlea by only 3 dB.

The solid lines represent the average shift of masking above 2.0 kHz (the *D*-values) in each of the 3 subjects investigated.

It is seen from Fig. 5 that the continuous lines are close to or below the interrupted line. The good agreement between the curves points strongly to the attenuation of sound transmission as the sole explanation for the antimasking effect provided by the stapedius reflex.

## DISCUSSION

The results from the present study showed that the low frequency noise masked high frequency sounds to a much greater extent in an ear without stapedius reflex than it did in an ear with normal stapedius reflex. The decrease of masking due to stapedius activity thus improved hearing in a wide frequency range up to at least 8.0 kHz,

probably even to the upper frequency range of hearing. A normal stapedius reflex masked threshold up to about 40 dB. The masking effect can wholly be explained by the attenuation of the low frequency sound provided by the stapedius reflex (Fig. 1).

It might seem surprising that the masking is unaffected by the stapedius reflex at frequencies below about 2.0 kHz. Two reasons for this finding can be given.

(1) In the frequency range below 2.0 kHz the stapedius reflex attenuates both the masker and the masked sound. Thus a stapedius reflex contraction is followed by an approximately equal decrease in the transmission of both the masker and the masked tone through the middle ear.

(2) Since masking close to the masker frequency grows slowly as the masker level is increased (see e.g. data by Blom & Lohr-Ehmer, 1959a) the attenuation of the masker affects masking less in this frequency range at greater distance from the masker.

## Functional significance of an antimasking effect

The traditional view of the stapedius reflex is that of an attenuator of transmission of low frequency sound in the middle ear. The attenuation amounts to about 20 dB (see Fig. 1). This agrees with earlier results (Borg & Lohr-Ehmer, 1973). The finding of this study consists of the effect of the stapedius reflex on hearing at high frequencies. The reflex attenuation of a low frequency masker improves hearing at high frequencies by up to 40 dB.

It has recently been shown (Borg & Lohr-Ehmer, 1973) that discrimination of speech is significantly impaired in ears with stapedius paralysis at levels above the normal hearing threshold. The antimasking effect shown in the present study offers a probable explanation for this finding: the strong low frequency attenuation of speech sounds do not mask the high frequency ones in the presence of the stapedius reflex. The properties of a functional stapedius reflex and speech characteristics thus seem to be well adapted. The significance of the stapedius reflex masking effect on speech discrimination has been suggested earlier on the basis of

animal was then prepared for electrophysiological recording: a platinum-iridium ball electrode was placed on the round window and fixed with dental cement.

Lesions were made at a point approximately  $\frac{1}{2}$  turn from the round window in the basal

The bony wall of the cochlea was thinned with a small diamond mill. The finely thinned wall was then removed carefully in pieces with a delicate pick, attempting to avoid damage to the periotic labyrinth. Final lesions of the endolymphatic membrane and cochlear vasculature were made with a fine microdissecting knife or an electrode insulated except for an exposed tip (approximately 5 microns in diameter) used to cannulize individual vessels. Most lesions were made in the scala media, two were restricted to the scala vestibuli and one to the scala tympani.

#### *Electrophysiological procedures*

A ear speculum coupled the external auditory meatus to an earspeaker (PDR 600). A  $\frac{1}{2}$ -inch condenser microphone with a calibrated probe was used to monitor the sound pressure level, 2 mm lateral to the tympanic membrane. Throughout the electrophysiological procedures, the gulla remained open.

Measurements of the cochlear microphonic (CM) and  $N_1$  response were made following intracranial preparation of the animal. These responses were evaluated immediately following lesion at various time intervals following lesion treatment. The one microvolt isopotential output for the CM was evaluated with a Radio Wave analyzer. Frequencies tested included 0.08, 0.20, 0.50, 1.0, 2.0, 6.0, 10.0, 20.0, and 30.0 kHz. The input-output (dynamic range) functions of the CM were examined for 6.0 kHz, except for Animal No. 1 in which this function was examined at 4.0 kHz. The  $N_1$  action potential was recorded as a series of 10 clicks presented at 10 per second. The clicks were generated by 2 V, 0.01 msec square wave pulses presented through the ear speaker. Both latency and amplitude of the  $N_1$  response were evaluated.

#### *Morphological procedures*

Following termination of the electrophysiological portion of the experiment, the vascular system of the animal was perfused with Prussian blue solution (See Axelsson, 1968, 1972, for details of this procedure). The temporal bones were then removed and cochlea fixed and stained (Millonig's buffered osmic acid). The cochleae were decalcified in 10% EDTA buffered to 7.4. Following washing, dehydration, and transfer to glycerol, the temporal bones were dissected with the aid of a dissecting microscope. Sections of the external wall, spiral lamina, and basilar membrane were mounted on slides and the vasculature, sensory neuroepithelium, and supporting structures were examined under light and phase contrast microscopy. The details of this new "soft-surface preparation technique" will be published elsewhere (Axelsson et al., 1973).

## RESULTS

Only clear changes in electrophysiological responsiveness and histopathological observations following surgery are reported. Omission of comment indicates that electrophysiological characteristics were unchanged following lesion and morphological features were characteristic of the normal guinea pig.

#### *Animal No. 1 (19)*

*Surgery:* Restricted scala media lesion. Little leakage of perilymph.

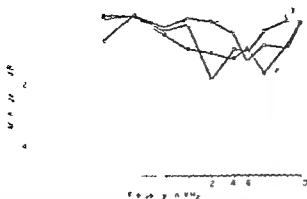
*Electrophysiology:* A fairly broad depression of the one microvolt CM contour was seen (Fig. 1). Frequencies from 0.50 through 20.0 kHz were affected by this lesion; higher and lower frequencies were normal. Immediately postoperatively, a maximum depression of 12 dB was seen at 4.0 kHz.  $N_1$  and input-output functions were affected in a similar manner to that seen in Animal No. 2—see Fig. 12.

Morphology was not available on this subject.

#### *Animal No. 2 (20)*

*Surgery:* Restricted scala media lesion. Little leakage of perilymph.





**Fig 1** Change in 1pV sensitivity contour following lesion restricted to scala media. The post lesion response of each animal (—) are compared with their pre-lesion level of response (---)

**Electrophysiology** See Fig 1 Greatest effect is on frequencies in the 60 kHz range Maximum sensitivity change was 13 dB immediately after the lesion It was stable during the 3 hours of observation Fig 2 shows dynamic input output functions at 60 kHz Shift in input output function is consistent with a sensorineural loss The function was shifted to the right and the point of nonlinearity occurred at a lower intensity above 1  $\mu$ V threshold Changes in N.

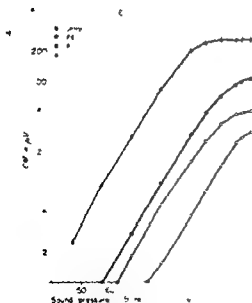


Fig. 2. Median and range of dynamic input-output function of the cochlear microphonic for three guinea pigs prior to lesion (control) and following scala media (GP 2 and 3) and scala tympani lesions (GP 6).

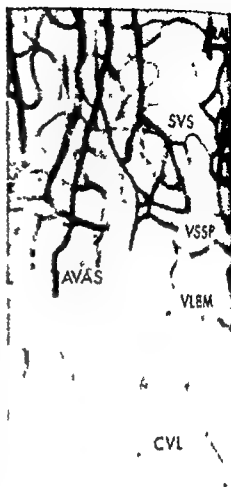


Fig. 3 Animal No. 2. Discrete lesion of the c

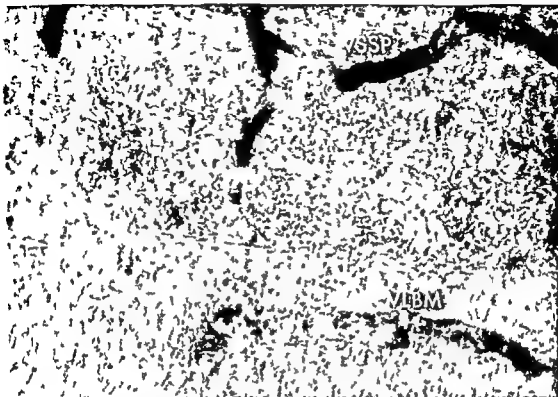
erient vascular contrast injection in  
arterioles (RAL) arteriovenous anastomoses  
the venules at the basal membrane (11)  
collecting venules (CVL)

potential are consistent with changes in the CM

**Morphology:** Damage was in a narrow segment of vessels of the spirals and the vessel of the spiral and affected only peripheral parts of the arterioles in scala vestibuli and anastomoses in scala media and cochlea in scala tympani (Fig. 3). Blood and debris were found in the external spiral sulcus (Fig. 4).

*Animal No 3 (21)*

Animal No 3 (21)  
Surgery Small penetrating lcs - 1st  
media



Animal No. 2. In the outer spiral sulcus between vessel of the spiral prominence (VSSP) and the basement of the basilar membrane (arrow) there is an

accumulation of blood and debris. VLBMs: the venules at the basilar membrane.

**electrophysiology** Restricted frequency range of the cochlear microphone contour 1) immediately following surgery. Dominant effect is in 20 kHz range (19 dB depression).



2 4 6 8 10 20 30  
Frequency (kHz)

Change in  $N_1/V$  sensitivity contour following lesion of the scala vestibuli. The post-lesion responses (animal —) are compared to the pre-lesion responses (—).

During the following hour sensitivity decreased further for low frequencies. The function then remained stable during the subsequent 4 hours. Fig. 2 shows dynamic input/output functions at 60 kHz. Changes were consistent with a sensorineural loss. Function was to the right and the point of non-linearity occurred at a lower intensity above the  $1 \mu V$  threshold. Changes in  $N_1$  potential were consistent with changes seen in CM.

**Morphology** Damage affected the stria vascularis which was penetrated in its most basal part in a very restricted area. Spiral prominence was also severed in a limited area whereas external spiral sulcus was unaffected.

#### Animal No. 4 (23)

**Surgery** Isolated scala vestibuli lesion with section of three radiating arterioles.

**Electrophysiology** The  $1 \mu V$  isopotential

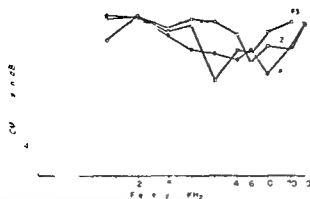


Fig 1 Change in  $1 \mu\text{V}$  sensitivity contour following lesion restricted to scala media. The post lesion response of each animal (—) are compared with their pre lesion level of response (---)

**Electrophysiology** See Fig 1. Greatest effect is on frequencies in the 60 kHz range. Maximum sensitivity change was 13 dB immediately after the lesion. It was stable during the 3 hours of observation. Fig 2 shows dynamic input-output functions at 60 kHz. Shift in input-output function is consistent with a sensorineural loss. The function was shifted to the right and the point of nonlinearity occurred at a lower intensity above 1  $\mu\text{V}$  threshold. Changes in  $N_1$

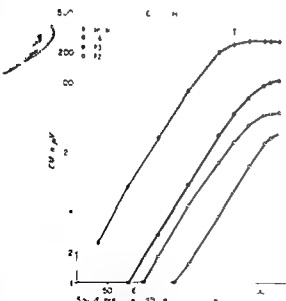


Fig 2 Median and range of dynamic input-output function of the cochlear microphonic for three guinea pigs prior to lesion (control) and following scala media (GP 2 and 3) and scala tympani lesion (GP 6)

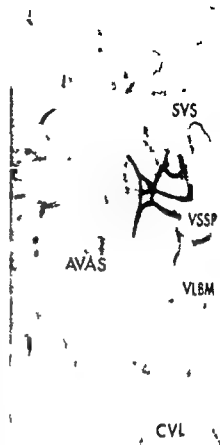


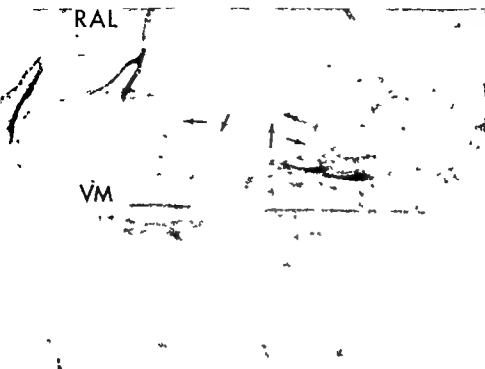
Fig 3 Animal No 2. Discrete lesion of the basilar turn only influences the vessel of the stria vascularis (SVS) and the vessel of the stria (VSSP). There is a narrow segment of the vessel (AVAS) and the venules at the basilar membrane (VLM) and the collecting venules (CVL).

potential are consistent with changes in the CM.

**Morphology** Damage was restricted to a narrow segment of the vessel of the stria vascularis and the vessel of the stria. The lesion affected only peripheral parts of the stria, including arterioles in scala vestibuli and anastomoses in scala media and scala tympani (Fig 3). Blood and debris were found in the external spiral sulcus (Fig 4).

#### Animal No 3 (21)

**Surgery** Small penetrating lesion of the scala media.

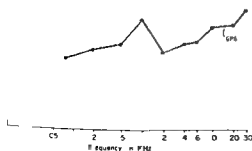


Animal No. 5 The scala vestibuli lesion (arrows) is accompanied by a segment of uninjected vasculature including all vessels peripheral to the lesion and the radiating arterioles (RAL) central to the lesion. The attachments of vestibular (VM) and the basilar (BM) membrane are unusually prominent probably due to accumulation of blood SVS stria vascularis

our is shown in Fig. 5. The greatest effect was at 4.0 kHz (13 dB) with an additional broad peak in the low frequency range with a maximum at 0.50 kHz. This occurred immediately

following the lesion and remained stable for the following 4 hours. A continuously decreasing change in the  $N_1$  response occurred during the 4 hours observation post lesion. Immediately following the lesion, the  $N_1$  amplitude decreased from 148  $\mu$ V to 104  $\mu$ V, then continuously decreased to 45  $\mu$ V.

**Morphology.** Lesion is restricted to the scala vestibuli. Three radiating arterioles are severed (Fig. 6). Fracture lines emanate from the operated region in both directions to the scala media. Attachments of Reissner's membrane both in spiral limbus and external wall were more prominent than normal, probably due to collection of blood corpuscles. Radiating arterioles at site of lesion were uninjected both centrally and peripherally. There is a segment of uninjected vessels corresponding to the lesion in the scala vestibuli, media and tympani.



Change in  $1/\mu$ V sensitivity contour following lesion related to scala tympani

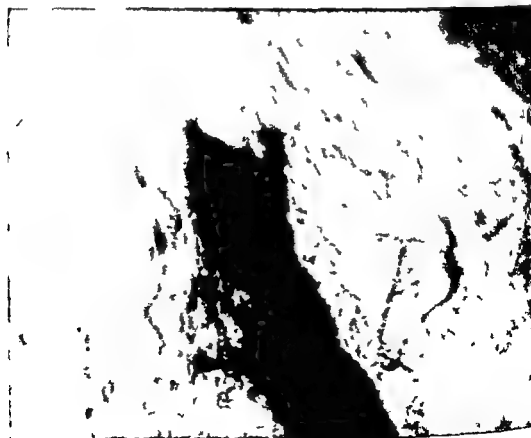
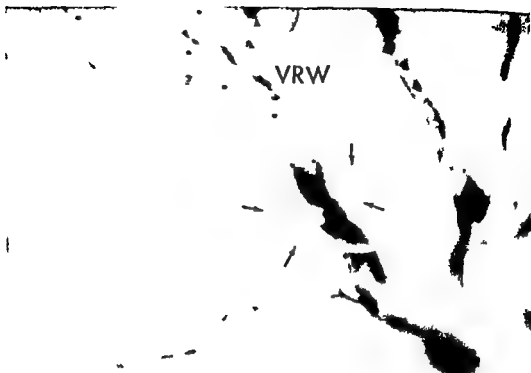




Fig. 1. Change in  $1 \mu\text{V}$  cochlear microphonic sensitivity at intervals during staged surgery and lesion of the scala media. See text.

#### Animal No. 5 (26)

*Procedure:* Restricted lesion of the scala vestibuli section of three radiating arterioles.

*Electrophysiology:* Fig. 5 shows the  $1 \mu\text{V}$  CM potential contour. A maximum deficit at 10 was seen following the lesion.

*Morphology:* Lesion is restricted to scala vestibuli with sectioning of three radiating arterioles (Fig. 7). A narrow segment of vessels corresponding to the lesion is uninjected by contrast in scala vestibuli, media and tympani.

8) There is moderate hemorrhage on the surface of the cochlea at the site of lesion. The segment of the basilar membrane in the middle of the cochlea wall appeared darkly stained.

#### Animal No. 6 (29)

*Procedure:* Isolated scala tympani lesion with sectioning of the vein of the round window.

9) perilymph leakage. Marked bleeding observed within 10 seconds.

*Electrophysiology:* Fig. 9 shows a marked

2) Animal No. 6 (A) Small lesion (arrows) only involving the vein of the round window (PRIF) in the part of scala tympani close to the round window. Contrast is found outside of the vessel lumen. (B) High magnification showing the hole in the bone of the scala tympani and the contrast in the vein of the round window.

and relatively broad low frequency effect in cochlear sensitivity occurred following lesion. The change in input-output (Fig. 2) and  $N_1$  change was consistent with a sensorineural hearing loss.

*Morphology:* Lesion is confined to the basal parts of the scala tympani and to the region of the vein of the round window. The vein, however, is injected by contrast (Fig. 10A, B). No blood and little if any contrast was observed outside the vessel lumen.

#### Further Electrophysiological Analyses

In an attempt to ascertain the amount of trauma caused to the cochlea by the surgical procedures, measures of the electrophysiological response were made at various times during the surgical procedure. Figs. 11 and 12 illustrate differences observed in the CM. The  $1 \mu\text{V}$  CM was little affected by the thinning, drilling and opening of the small fenestra; however, following widening of the fenestra the CM decreased in sensitivity. Fig. 12 on the other hand shows a moderate decrease in the linearity of the CM before the fenestra was widened. Moreover, there is truncation of the CM after widening and sealing. This indicates the greater sensitivity of the input-output function to cochlear changes.

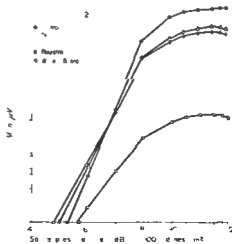


Fig. 12. Change in input-output function of cochlear microphonic at intervals during staged surgery and lesion of scala media. See text.

## DISCUSSION

Reservations must be placed upon any conclusions drawn from this study. Of primary concern is the small sample size of subjects and observations upon which the results of this paper are based. In addition, both the electrophysiological as well as the morphological techniques employed have certain limitations. However, we suggest that an approach using both electrophysiological and morphological techniques may permit a better evaluation of the normal or pathological cochlea than either technique alone. The injection of Prussian blue in the cochlear vasculature provides information on the functional state of the vessels following these gross lesions.

In the literature no similar experiments comparing morphological and electrophysiological effects of limited lesions to the cochlear are available. Certainly, many different modes of influencing the cochlea in order to resemble clinical conditions with sensorineural hearing losses have been attempted. Only a few of these have been confined and performed on the external wall (Stahle et al., 1972; Sugar et al., 1972; Stahle & Sugar, 1973; Axelsson & Hallén, 1973). These studies did not evaluate the functional state of the cochlea. Tasaki et al. (1952) examined the electrophysiological effects of lesions placed in the various scalae of the guinea pig. They saw no change under any condition except following the introduction of foreign material into the scala media sufficient to press upon the basilar membrane. Their recordings were made via differential electrodes introduced to the scalae of the guinea pig cochlea. The discrepancy between the cochlear microphonic findings of that study and the present investigation may in part be based upon differences in recording procedures. No morphological examination of the cochleae was made by Tasaki et al. (1952).

Certain findings were clear in the present study. In a number of cases in which CMs were recorded at various stages while the coch-

lear wall was thinned, picked and/or torn, we saw little or no effect on the CM response (Fig. 11). In general the most important factor on activity recorded from the window appears to be the presence of a following an opening into any of the scalae regardless of location or size of the lesion. A correlation did exist between the location of the lesion and the extent of reduction in the microphonic. It appeared as though the media lesion (3 cases) are most specific in terms of the frequency band that was affected than scala vestibuli lesions (3 cases). We suggest that this observation indicates that the primary influence of these lesions is not only hydrodynamic in nature. There may be a local change in mechanics of the basilar membrane or a local change induced by the affected vasculature.

The mechanical lesions had little or no term influence on the organ of Corti as seen by light- and phase contrast microscopy. No changes were seen in the sensory supporting structures or vessels of the lamina. In the short time studied in this experiment, the only morphological changes were those mechanically induced at the site of the lesion.

The most interesting finding in the present material is the effect of the various locations and location of the lesion in the cochlea and on the different vessels on cochlear microphonic activity. In the two lesions affecting the scala vestibuli, three large radiating arterioles were severed. The segment of the vasculature peripheral to the lesion was un.injected in all three scalae. Further, the segment of the radiating arterioles were also uninjectable centrally to the lesion from the round window. This is consistent with the previous findings (Axelsson & Hallén, 1973) in which a 'reflex closure' of the radiating arterioles was seen. We suggest that such a reflex closure may serve to limit effects of haemorrhage on injured cochlear structures and on the CM. Moreover, the segmental reflex closure of the radiating arterioles may account for the



† Animal No 4 Sensory and supporting structures basilar membrane immediately opposite lesion external wall No morphological changes observed

(400  $\mu\text{m}^2$ ) OHC 1-3 first, second and third row outer hair cells IHC - inner hair cells

relatively restricted electrophysiological changes observed like some lesions (e.g. of the round window), the cochlear response was not depressed throughout all frequencies examined. In the scala tympani lesion, the vein of the round window was severed by surgery. This vein received most of the collecting venules in the scala media in the most basal part of the cochlea. Surprisingly, in spite of the considerable initial bleeding, the subsequent injected contrast medium filled within the vessel lumen. This may indicate that during the 4-hour electrophysiological observation period following lesion some process occurred either in the vessel wall or soft tissues leading it to functionally 'seal' this vessel. The pronounced electrophysiological changes may be attributed to the proximity of the lesion to the recording site. It can also be assumed that the size of the lesion has caused a considerable leakage of perilymph. Otherwise, it is unlikely that this restricted vascular lesion

with remaining vessel continuity on the venous side beyond the capillary areas should cause such a pronounced effect.

Again from vascular considerations, it is surprising that the very superficial or deeper, but in all cases very limited vascular lesions, in the scala media were reflected in such severe electrophysiological changes. We suggest this can only be explained on the basis of local mechanical effects—such as hydrostatic changes or distortion of the soft tissues of the scala media.

In conclusion, the present investigation demonstrates a combined approach of evaluating the effects of mechanically induced lesions with the aid of electrophysiological and morphological measurements of both CM, N<sub>1</sub>, and evaluation of vessels and sensory and supporting structures of the cochlea. Our findings indicate that these restricted lesions result in restricted changes in the electrophysiological and morphological state of the cochlea. This is encouraging



in that similar to the findings of the previous anatomical study it appears as though these types of restricted damage apparently are restricted both initially (functionally and structurally) and at longer times (structurally) in their effects. We did not find a clear, orderly and detailed correlation between size and location of lesion and the change in cochlear activity. Ongoing experiments both in animals assessed soon after surgery and more long standing experiments are needed to determine to what extent hydrodynamic changes, segmental vascular injuries and other processes influence the electrophysiological and morphological state of the cochlea.

### ZUSAMMENFASSUNG

Mechanisch wurde die äussere Wand der Cochlea des Meeresschweinchens geringfügig beschädigt. Man untersuchte die Wirkung dieser Schaden auf Blutgefässe, Haarzellen und das tragende Gewebe elektrophysiologisch und mittels histopathologischer Technik. Diese Methode erscheint vielversprechend. Die begrenzten Schaden resultierten in einer begrenzten Veränderung der Electrophysiologie sowie der Morphologie der Cochlea. Häufige und regelmässige Veränderungen von Grösse und Lage des Schadens und der cochlearen Aktivität wurden nicht gefunden. In diesem Kurzzeit Experiment sind keine morphologischen Effekte am Cortischen Organ beob-

achtet worden. Der Schaden begrenzte ein schmales Gebiet der äusseren Wand und nicht der dortigen Blutgefässe.

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## POSTNATAL DEVELOPMENT OF ENDOCOCHELEAR POTENTIAL AND STRIA VASCULARIS IN THE CAT

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(Received January 9, 1974)

**Abstract** The postnatal development of the stria vascularis and endocochlear potential was studied in the cat.

Maturation of the stria was completed at about the 5th postnatal day. The development of the basal stria was characterized by its reduction in size and shape and by reduction of content of glycogen particles. The intermediate and marginal cells developed into complex structures with a large number of cytoplasmic compartments, containing a sinking conglomeration of mitochondria. Postnatal development of EP paralleled that of the stria vascularis. The increase in EP's values from (about 8.8 mV) to the 27-29th day (about 75.7 mV) followed an S-shaped curve.

The endocochlear potential (EP) of mammalian species is an electrical polarization (endocochlear potential) of 80-90 mV positive with respect to the perilymph of the scala vestibuli and tympani (Schmidt & Fernandez, 1963; Davis et al., 1952). The ionic composition of the perilymph is unique,  $K^+$  and  $Na^+$  concentrations approach that of intracellular fluids while the concentration in the perilymph resembles that of extracellular fluids (Smith et al., 1954). The processes underlying generation of the EP and maintenance of electrolyte balance in the labyrinthine fluids are still poorly understood. Experimental evidence indicates that EP is dependent on the metabolic activity of the stria vascularis and that it is not directly related to the ionic composition of the labyrinthine fluids (Davis et al., 1955; Tasaki, 1966; Konishi et al., 1966). Developmental studies have advanced our understanding of the relation between EP, stria vascularis and ionic

composition of labyrinthine fluids. Relevant data on postnatal maturation of labyrinthine fluids and EP have been reported by Boshier & Warren (1971). In the white Wistar rat, the ionic concentration of  $K^+$ ,  $Na^+$ , and  $Cl^-$ , in both endolymph and perilymph, reached near adult values at about the eighth postnatal day, while EP remained below +20 mV until the eleventh day. They found also that maturation of the stria vascularis and organ of Corti, excepting the Claudius and Boettcher cells, paralleled that of the labyrinthine fluids. Thereafter, EP increased rapidly, reaching adult value (-92 mV) at about the sixteenth postnatal day. They hypothesized that the rapid development of EP between the eleventh and sixteenth postnatal day results from "a rapid increase in the electrical resistance of some portion of the membranes bounding the endolymphatic space at this time". They also mentioned the alternative interpretation that EP development may be associated with maturation of its generator. Schmidt & Fernandez (1963) and Ånggård (1965) have related the development of EP to the postnatal maturation of the stria vascularis, an opinion supported by the work of Kikuchi & Hilding (1966). Their investigation showed, for the mouse, a progressive ultrastructural growth of the marginal, intermediate and basal cells of the stria vascularis from birth to the eleventh postnatal day.

In order to investigate further the relation between postnatal development of EP and stria

This work was supported by Public Health Service Grants NS 00682 and NS 06809.

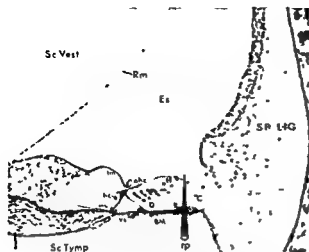


Fig 1 Cochlear duct of the first turn of an adult cat showing the position of the micropipette (*rp*) for recording resting potentials. *Sc Vest*, Scala vestibuli, *Sc Tym*, Scala tympany, *Es*, Scala media

vascularis, an experiment was designed in which EP was measured in kittens of known age and their stria vascularis examined with both light and electron microscopy. The cat was selected as the experimental animal because this species has been and is extensively used in auditory physiology, while few data are available on the functional and morphological development of their cochlea (Rose et al, 1957, Pujol & Marty, 1970, Romand, 1971).

## MATERIALS AND METHODS

Observations were made on 188 kittens and 24 adult cats. All kittens were born in our animal quarters and their ages, ranging from three hours after birth to 142 days old, were known at the time of EP measurement. Animals weighing less than 100 g during the first postnatal day were considered premature and discarded. Each kitten was assigned at birth to a group of animals to be tested at a specific age, but 38 out of 188 animals either died before or during testing or were discarded after failure to measure EP.

For recording EP the animal was anesthetized with sodium pentobarbital (30 mg/kg) given intraperitoneally. After tracheotomy and fixa-

tion of the head, the round window was exposed by opening the mastoid process in the area formed by the lambdoid ridge and the posterior border of the external auditory meatus. Although the basilar membrane can be visualized and punctured through the round window membrane, we decided to remove it in order to obtain a clear visualization of the basilar membrane. It should be mentioned that the removal of the round window membrane results in leakage of perilymph and the question has been raised as to whether this procedure may alter the generation of DC resting potentials in both Corti's cells and the scala media. There is no evidence to support this in the case, since our measurements are in agreement with those of others working with round window closed (Butler, 1965) or a large opening in scala media (Tasaki et al, 1954) are about the same magnitude as that obtained by those working with round window closed (Fernandez et al, 1967). By means of this exposure, the recording micropipette was inserted under high magnification without disturbing the circulation of the spiral lymph, which in the kitten are large relative to those in the adult cat. The recording electrode penetrated the basal turn between the spiral lamina and the spiral ligament where the epithelium of the scala media is formed by the Hensen's cells. More laterally by the Claudius' cells or the Boettcher's cells (Fig 1). Histochemical studies, however, revealed that some preparations were also made through Deiters' sensory cells (see Fig 13).

Micropipettes filled with 3M KCl or 0.1M NaCl (impedance 5–8 megohms) were used for recording DC potentials. The micropipettes were mounted on a hydraulic micromanipulator which permitted reposition and placement of the electrode's tip at any desired location on the basilar membrane. An Ag/AgCl electrode connected to the micropipette to a Keithley electrometer (Model 250). The micropipette was inserted slowly through the basilar membrane into the organ of Corti and then into the scala media. The DC potentials of the organ of Corti and scala media were read directly on the electrometer.

and permanent records were obtained by feeding the output of the electrometer into an

Dynograph. In some animals measurements were made in one ear only while in others measurements were bilateral.

Twenty-three kittens—representing ages from 10 to 90 days old—were killed after completion of P measurement by intravital perfusion of Bouin's fixative and their temporal bones were removed and processed for histological studies with light microscopy. For electron microscopy studies 26 kittens of ages ranging from three hours to 30 days old were used. The cochlea was fixed intravitaly and the sections prepared for electron microscopy as previously described elsewhere (Hinojosa, 1971).

## RESULTS

### Histological studies

**Postnatal development of Corti's organ.** Some stages of the postnatal development of Corti's organ are depicted in Fig. 2. A premature kitten (Fig. 2A) shows a striking configuration of densely packed cells lining the inner wall of the cochlea. This embryonic structure referred to by Boettcher (1863) as the Kolliker's organ disappears progressively from the base to the apex in about 25 days after birth.

The postnatal development of the epithelium over the pectinate zone of the basilar membrane of the first turn was studied in detail. It was through this area that the microelectrode for recording EP was advanced into the scala media. In the newborn kitten the Deiters' cells are columnar shaped. Their apical diameters are about  $10\ \mu$  and  $17\ \mu$  respectively (Fig. 2B). These cells reach maturation in 20–25 days (Fig. 2F). Lateral to the Hensen's cells the outer membrane of the basal turn of the new-born kitten is lined by Boettcher's cells. As shown in Figs. 2A–2E the Boettcher's cells—located between Hensen's and Claudius' cells—are in contact with the endolymph. The Claudius' cells are apparently located in the outer sulcus although some may be interposed between Hensen's and Boettcher's cells.



Fig. 2. Photomicrographs of the cochlea in a premature kitten at 15 days of age.

At birth there is a large number of cells at the base of the organ of Corti in contact with the outer wall of the cochlea (Fig. 4). These cells are in contact with the apical surface of the outer membrane of the basilar membrane (Fig. 4). In some areas the basal plasma membrane is more than 20  $\mu$  thick (Fig. 4). By Ishiyama (1968) about 20 days after birth the cells grow rapidly (Figs. 2F, 2G). Boettcher's cells are located by the outer wall (Fig. 2H). The cells show little variation in height. The cells can be seen underneath the outer membrane of the basilar membrane. The cytoplasm of the Claudius' cells is



Fig. 3. Ultrastructure of Boettcher's cells in a one day old kitten. Their junctional complex, lateral and basal plasmalemmae are illustrated in Figs 4, 5, and 6 respectively.  $\times 5814$ .

Fig. 5 Middle portion of the lateral plasmalemma. The intercellular space in this area measured 25 Å.

Fig. 6 Basal area of the lateral plasmalemma. Numerous infoldings. The intercellular space about 160 Å.  $\times 21450$ .

The com-  
adherens

segment, the cells of Hensen, Claudius and other exhibit no cell multiplication or cell division.

The spiral vessel of the basilar membrane shows a striking developmental feature consisting of a rapid reduction of its cross-sectional area from about  $500\text{--}600\ \mu^2$  at birth (Fig. 2B) to about  $15\text{--}30\ \mu^2$  within the next 10 days (Fig. 2C). The large areas of the vessel may be related to the metabolic requirements of the developing organ as mentioned by Lawrence (1971). The cells lining the tympanic side of the basilar membrane form a thick layer in the premature newborn kitten (Figs 2A, 2B), but within a few weeks the cells are considerably reduced in size (Fig. 2E).

**Postnatal development of stria vascularis.** The stria vascularis of the newborn kitten (Fig. 7) differs considerably from the stria of a 30-day-old or adult animal (Fig. 12). The basal cells of the newborn are large and cuboidal with a pale basophilic cytoplasm containing a few organelles and marginally near the cells' walls and abundant glycogen particles (Fig. 8). These particles were identified using the criteria of Revel (1963) and Revel (1964), that is, in sections stained with lead hydroxide, with or without uranyl salts, the glycogen appears as round or oval particles of clear cut margins and high electron density. In the basal cells the particles are of the particulate type (about  $300\ \text{\AA}$  in diameter) forming a substructure ( $30\text{--}40\ \text{\AA}$ ) with dense cores (Fig. 8, inset). Ribosomes are smaller particles (about  $150\ \text{\AA}$ ) and stain darker than glycogen (Fig. 9). The nucleus of basal cells is large and irregular in shape. In the kitten and adult cat as well the basal cells may be found juxtaposed between marginal cells and cells of the inner zone of the Reissner's membrane. The size, shape and ultrastructure of the cells lining the attachment zone of the Reissner's membrane of the kitten are similar to those described in the adult by Hinojosa & Rodriguez-Echandia (1966). The postnatal development of basal cells is characterized by a progressive shrinking (Figs 7, 10–12) and a diminution of glycogen particles (Fig. 9) so that in the 25–30

day old kitten, the cells exhibit the laminar feature of the matured cell with a large nucleus forming a striking prominence (Fig. 12).

The postnatal development of marginal cells is illustrated in Figs 7, 10, 11 and 12. At birth, the apical plasmalemma in contact with endolymph exhibit microvilli, coated vesicles and a fully developed junctional complex (Fig. 7). In some micrographs, however, the junctional complex shows a gap at the level of the zonula occludens. The basal plasmalemma presents shallow infolding which increases considerably in size and number during the first 25 postnatal days (Figs 10–12). The cytoplasm contains numerous vesicles and other organelles generally found in mature cells. In the course of postnatal development the cells increase in size from about  $14\ \mu$  in height to about  $24\ \mu$  by the 30th day and to  $37\ \mu$  in the adult. A striking developmental feature is the accumulation of numerous mitochondria in the interdigitations of the basal plasmalemma.

The intermediate cells of the newborn kitten exhibit a moderate number of organelles which gives to their cytoplasm a light appearance in contrast to the even lighter aspect of the basal cells and heavy density of the marginal cells. The most noticeable developmental feature of intermediate cells was a considerable increase in the number of interdigitations of their plasmalemma (Figs 7, 10–12).

In the kitten no basal lamina was ever found between basal cells and spiral ligament or underneath the marginal cells. The absence of basal lamina in this location holds also true for the adult cat (Kikuchi & Hilding 1966, Hinojosa & Rodriguez-Echandia, 1966). In the newborn mouse, however, Kikuchi & Hilding (1966) found a basement membrane underneath the marginal cells. They assumed that this feature strongly supported the opinion that the marginal cells are of ectodermal origin (Engstrom et al., 1955; Weibel 1957). As in the adult, the stria capillaries of the newborn kitten are surrounded by an endothelial basal lamina which is continuous with that of capillaries of the spiral ligament (Fig. 10). The endothelial basal lamina of

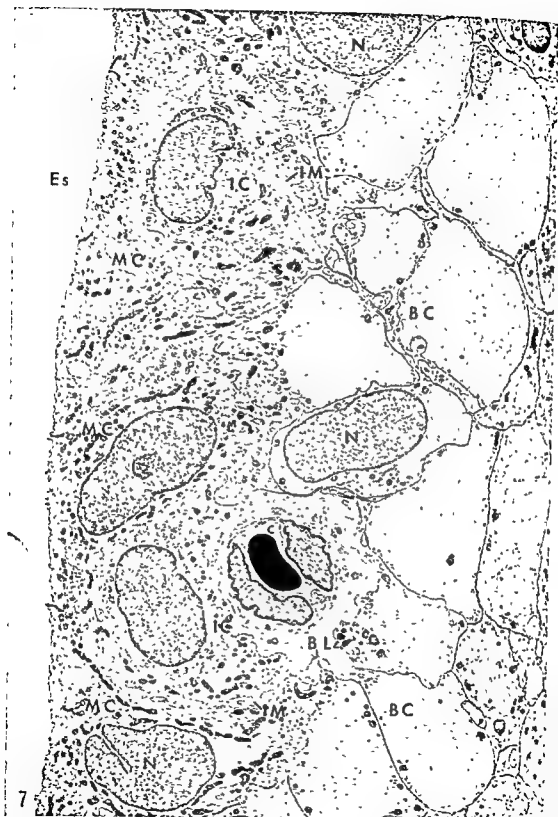


Fig 7. Electron micrograph of the stria vascularis (Basal turn) of a kitten three hours after birth. The marginal cells (MC) show a 'fence-like' appearance.

the basal cytoplasm. The intermediate cells (IC) show a

pale cytoplasm with numerous organelles. The digitations with the marginal cells can be seen. The cells (BC) are large, their cytoplasm appears granular; it contains a few organelles located at the periphery.  $\times 4195$ .

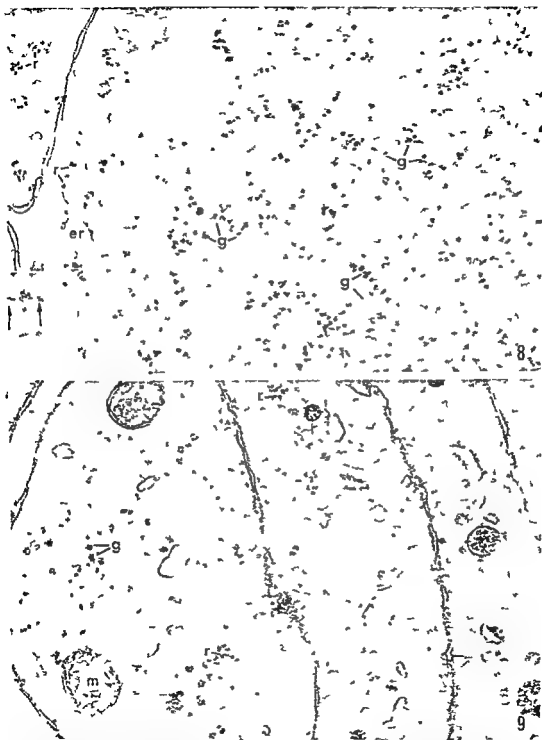


Fig. 8 Basal cells of the stria vascularis of a newborn kitten containing abundant glycogen particles (g). Inset showing glycogen particles at high magnification showing the cellular substructure (arrows). er Endoplasmic reticulum. r Ribosomes. 47 652 inset  $\times 85\,272$ .

Fig. 9 Basal cells of the stria vascularis of a 30 day old kitten showing few glycogen particles (g). m Mitochondria. r Ribosomes.  $\times 47\,652$ .





Fig. 10. Ultrastructure of the stria vascularis in a 10 day old kitten. The most prominent change up to this age was the reduction in size of the basal cells (BC) and increase in density of their cytoplasm. This electron micrograph

illustrates the extension of the basal lamina (BL) between the blood vessels (C). Arrow points toward extension of the basal cells between the marginal cells (MG).



1 Ultrastructure of the stria vascularis in a 20 day old animal. The maturation of the stria are close to that noted by an adult animal - 4 377

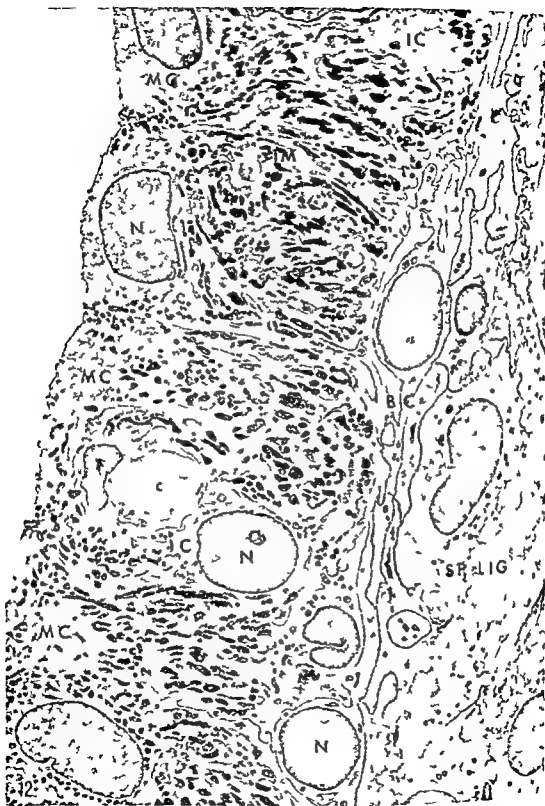


Fig 12 Stria vascularis of a 30 day old kitten. The ultrastructure is similar to that of the adult cat. 3556

cat also exhibits expansions penetrating between the stria cells (Figs 7, 10-12, *IMJ*)

### Physiological studies

Serial sections of the cochlea showed that the best puncture for recording DC potentials was made at 4-5 mm from the basal end of Corti's organ. In some specimens the puncture was made at the level of Boettcher's cells while in others the micropipette penetrated the basilar membrane at the level of Deiters cells (Fig 13). The penetration of Corti's organ at any of these levels was associated with the recording of a negative DC voltage which changed polarity when the electrode entered the endolymphatic space (Fig 14). The negative potential of Corti's organ as a function of the animal's age is presented in Fig 15. There was a considerable variation on the measurements, but at birth the negative DC voltages of about the same value as those of the adult cat were obtained (Fig 14). The variation among measurements may reflect factors such as the speed of penetration, cell damage or physiological conditions of the animal. The potential of Corti's organ could be held for a short time before waning to zero, further advancement of the micropipette frequently registered a negative DC voltage again or a positive voltage. The latter indicated that the electrode penetrated the endolymphatic space. Withdrawal of the micropipette or a second penetration through the same path failed to register the negative potential of Corti's organ, but it could be recorded again by puncturing the basilar membrane at a fraction of a millimeter away from the previous hole. On the other hand, the cochlear potential could be recorded for long periods without decay and repetitively after puncture through the same path. The data on the endocochlear potential as a function of age are presented in Fig 16. The early growth from birth to about the 27-29th postnatal day was a striking feature. The value at birth (within 24 hours) was  $8.8 \pm 4.3$  mV  $\pm$  S.D.,  $N=10$ ) and at the 27-29th postnatal day its value was  $75.7 \pm 8.3$  mV ( $n=11$ ). The growth of EP from birth to maturation ex-

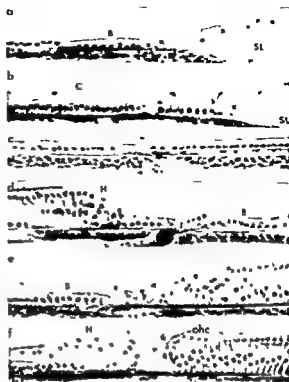


Fig 13 Samples of micropipette paths. The age of the kittens of a through f were 6, 28, 38, 9 and 22 days old and the negative dc polarization was 97, 80, 54, 80, 81 and 80 mV, respectively

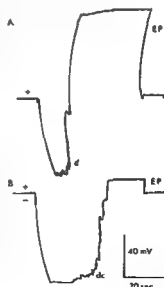


Fig 14 Cochlear DC potentials of an adult cat (A) and a newborn kitten (B). *dc*, Negative potential recorded in Corti's organ. After waning the electrode was advanced into scala media reestablishing the endocochlear potential (*EP*)

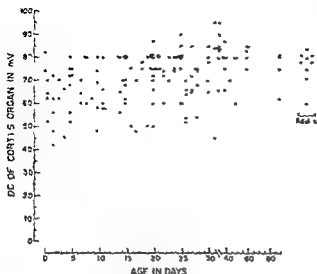


Fig 15 The negative resting potential of Corti's organ as function of age. Each point represents maximum value obtained per one ear ( $N = 161$ )

hibited a rate of increase of about 1.7 mV per day up to the 9th postnatal day. Then the rate increased to 3.5 mV per day until the 20th, followed by a slow rate of about 1.8 mV per day until maturation. In the adult cat the value of EP was  $80.4 \pm 7.77$  mV ( $n = 24$ ).

## DISCUSSION

*Postnatal development of Claudius' and Boettcher's cells.* The function of the Boettcher's cells

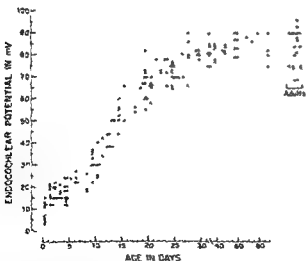


Fig 16 The endocochlear potential as function of age. Each point represents maximum value obtained per one ear ( $N = 239$ )

is unknown although ultrastructural (Katz et al., 1970) and histochemical (Katz & Plester, 1962; Rauch, 1964; Ishii & Batschelet, 1965) studies suggest that the cells may be provided with absorptive and/or secretory function. In our opinion, this opinion receives support from the fact that during development their apical processes are in contact with the endolymph and are provided with microvilli and a junctional complex.

An important developmental aspect of Claudius' and Boettcher's cells is the maturation lag that of other cells in the organ. Similar observations have been made in the rat (Bosher & Warren, 1971) and in the cat (Ånggård, 1965). This developmental lag raises the question whether the immature Boettcher's cells represent an area of high electrical resistance as postulated by Bosher & Warren (1971) to account for the gradual development of EP. In our opinion, this is not the case because the cells exhibit at birth a resting potential of the same magnitude as the adult, suggesting that the boundaries between either mature or immature cells might have low resistance. The solution of this problem requires measurements of the resistance across the organ in the kitten.

*Postnatal development of the stria vascularis.* The maturation of Corti's organ and stria vascularis proceeds orderly from base to apex in the cat and other species (Bast & Anson, 1947; Ånggård, 1965; Kikuchi & Hilding, 1966; Bosher & Warren, 1971) although Larsell et al. (1962) reported that in the opossum, the maturation proceeds from the upper basal and lower apical turn in a basal and apical direction.

At birth the marginal, intermediate and basal cells of the stria vascularis of the cat are well differentiated from the tectorial ligament. All changed in size and shape when they reached maturation at about 20 days after birth. One striking developmental feature of the stria vascularis is the progressive change of the "immature basal cell" in size, shape and cytoplasmic content. The functional significance of these changes is not clear. The intermediate and marginal cells develop into complex

a large number of cytoplasmic compartments containing a large conglomeration of mitochondria. These morphological features suggest that the structure may be involved in the transport of fluids and ions. That the stria is a major route of active ion transport has been indicated by biochemical methods (Matschinsky & Talmann, 1970) and that the stria plays an important role in the maintenance of ion concentration in the endolymph has been emphasized in the past. The relation between the composition of the stria and ionic composition of the endolymph, however, has been worked out only in the rat by Boshier & Warren (1971). In this species the chemical maturation of the stria parallels the histological maturation of the stria vascularis, tectorial membrane and Corti's organ. Maturation occurs at the 11th postnatal day, although Claudius' cells lag their maturation for seven additional days.

*Negative potential of Corti's organ.* Our observations support the opinion that it reflects the negative intracellular potential common to all cells (Bekesy, 1952; Tasaki et al., 1957; Davis, 1957; Dallos, 1968). The electron microscope provides convincing data that the junctional complex runs through the area occupied by Claudius', Deiters', Claudius' and Hensen's cells. It should record the intracellular potential of all cells found in the path of the microprobe. Their impalement seems unavoidable since the intercellular space between them is of the order of around 160 Å. Although these cells are immature at birth, the records demonstrate that their membrane potential was fully developed.

*Postnatal development of endocochlear potential.* The postnatal development of EP in the cat followed the general pattern found in the opossum (Schmidt & Fernández, 1963), rabbit (Ånggård, 1965) and rat (Boshier & Warren, 1971). The rate of growth, however, varies from one species to another. In the rat and mouse (Boshier & Warren, 1971; Schmidt & Fernández, 1963), growth occurs between the 10th and 15th postnatal day while in the opossum, rabbit and

cat the EP grows to maturation beginning with the first postnatal day.

Regarding the mechanism underlying maturation of EP, Boshier & Warren (1971) proposed that it may be related to the development of low electrical resistance of some portion of the membranes bounding the cochlear duct. It is tempting to consider the Boettcher's cells as one of these areas since they are in contact with the endolymph for about four weeks. Furthermore, their development and that of Claudius' cells parallels the development of EP, but it is not clear whether these events are related.

The opinion that the development of EP is associated with the maturation of the stria vascularis is supported by the observations of Schmidt & Fernández (1963), Ånggård (1965), Boshier & Warren (1971) and our observations. Tasaki & Spyropoulos (1959) located the generator of EP in the stria vascularis and the striking changes occurring in this structure during postnatal development strongly indicate that the progressive increase in magnitude of EP up to the 27 days old kitten is related to maturation of its generator in the stria.

## ZUSAMMENFASSUNG

Die Entwicklung der Stria Vascularis und des endokochlearen Potentials nach der Geburt, wurde bei jungen Katzen untersucht. Die Stria war ungefähr am 25. Tag nach der Geburt ausgereift. Die Entwicklung der Grundzellen wurde dadurch ausgezeichnet, dass sich die grossen Zellen mit reichlichem Zellplasma in Grösse und Form zu kleinen abgeflachten Zellen verringerten. Die mittleren und äusseren Zellen entwickeln sich zu komplexen Bestandteilen mit zahlreichen Zellplasmaabteilungen, die eine beachtliche Anhäufung von Mitochondrien enthalten. Die Resultate zeigen eine Parallelentwicklung zwischen dem endokochlearen Potential und der Stria Vascularis nach der Geburt. Die Zunahme der endokochlearen Potentialwerte von der Geburt (ca. 8.8 Millivolt) bis zum 27. bis 29. (ca. 75.7 Millivolt) beschreibt eine S-förmige Kurve.

## LIST OF ABBREVIATIONS

BC, Basal cell  
BL, Basal lamina  
BM, Basilar membrane  
B, Boettcher's cell  
C, Claudius cell

D, Deiters cell  
 Es, Endolymphatic space  
 H, Hensen's cell  
 IC, Intermediate cell  
 ihc, Inner hair cell  
 IM, Interstitial membrane  
 MC, Marginal cell  
 N, Nucleus  
 ohc, Outer hair cell  
 p, Pericyte  
 Rm, Reissner's membrane  
 SL, Spiral lamina  
 SP LIG, Spiral ligament  
 SV, Stria vascularis  
 tm, Tectorial membrane  
 vs, Spiral vessel

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## COMPARISON OF COCHLEAR MICROPHONIC POTENTIALS FROM ALBINO AND PIGMENTED GUINEA PIGS

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(Received January 9 1974)

Recent behavioral tests would seem to indicate auditory thresholds of albino guinea pigs are significantly lower than the thresholds of pigmented guinea pigs. In the present study, cochlear microphonic (CM) sensitivity functions were recorded from round windows of these two groups of anesthetized guinea pigs (*Cavia procellus*) as one measure of hearing performance. For 15 of 20 tested frequencies between 70 and 7000 Hz, the mean CM sensitivities of albino animals were not significantly different from mean CM sensitivities of 6 pigmented animals. The maximum cochlear potential magnitudes obtainable at frequencies (100, 1000 and 4000 Hz) from each group also were found statistically alike. It is concluded that there is no difference between the tested pig strains with regard to the measured parameters of cochlear microphonic potential.

Guinea pigs have been commonly used laboratory subjects for almost all aspects of auditory research. Most of these animals undoubtedly are of the albino variety, but it is conceivable that some investigators have studied mixtures of albino and pigmented types without recognizing the fact in the subsequent reports. Thus, the reader cannot be certain which variety was used when the guinea pig type was not specified. This point could be important were the behavioral auditory threshold measurements of Nuttall (1973) to be confirmed. In that study the albino guinea pig's hearing threshold was compared with the pigmented guinea pig's

threshold. At all frequencies tested, Nuttall reported that the albino animals displayed significantly better hearing (lower thresholds) than the pigmented group.

The behavioral testing scheme which was used in the Nuttall study was first reported by Anderson & Wedenborg (1965) and later used by Ernstson (1972). The method, simply stated, was the conditioned suppression of shivering due to cold. An alteration or interruption of a regular shivering pattern could be induced when a tone, followed by a shock, was presented to the animal. Shivering appears to be a good choice for a behavioral parameter because it may not require learning by the guinea pig, whereas learned tasks, such as the water licking described by Heffner et al. (1971), can require long training periods.

However, well shiver behavior compares to other behavioral methods for auditory threshold testing, the results reported by Nuttall (1973) indicate that it may not be valid to include both albino and pigmented strains together in studies of the hearing mechanism, e.g. in cochlear electrophysiological studies. The present report addresses itself to an examination of the cochlear microphonic potential (CM) from the inner ear in order to determine if the CM sensitivity (isopotential) function or the maximum CM magnitude (indicators which may have a direct relationship to the functioning endorgan) differ between the two strains of guinea pig.

This work was supported by Public Health Service Grant



Table I The range of the sound intensities required to produce 10  $\mu$ V from each of the two

Frequency, kHz		0.07	0.1	0.2	0.3	0.4	0.5	0.6
Albino data range dB re 10 <sup>-1</sup> N/m <sup>2</sup>	Max	-2	-4.7	-6.8	-12.0	-13.7	-19.0	-19.7
	Min	-8.6	-13.7	-20.8	-20.6	-23.3	-27.0	-30.1
Pigmented data range dB re 10 <sup>-1</sup> N/m <sup>2</sup>	Max	+1.8	-2.3	-9.5	-14.1	-21.2	-24.8	-24.6
	Min	-10.7	-15.3	-19.0	-23.7	-26.2	-31.9	-33.1

## METHODS

A total of 12 guinea pigs (*Caia procellus*) of both sexes, with body weights between 250 and 350 g were used in this study. The animals were divided into two groups, the first containing 6 pigmented animals (from Hilltop Lab Animals, Scottdale, Pa., 15683) and the second having 6 albino animals (4 from an established colony at Kresge Hearing Research Institute, 2 from Camm Research Institute, Wayne, N.J., 07470). All pigmented guinea pigs had dark colored eyes as well as patterned or solid colored fur. Each of the 12 animals exhibited a good pinna (Preyer) reflex to sudden loud sounds.

The animals were deeply anesthetized with allobarbitol (100 mg/ml) and urethane (400 mg/ml) anesthetic administered intraperitoneally (0.8 cc/kg). Respiration was maintained artificially by means of a tracheal cannula and heart rate was monitored for the duration of the experiment. A conventional postauricular surgical approach on the left side was used to expose the round window membrane of the cochlea. After fastening the head in a head holder, the left auricle was removed, leaving a ring of cartilage at the meatus, to facilitate connection of a sound speculum.

Sound intensity, in the closed field system, was determined with a calibrated probe tube microphone (Western Electric 640 AA) at a point approximately 3 mm from the tympanic mem-

brane in the external meatus. Cochlear phonic potentials (CM) picked up by a bead electrode on the round window, amplified 1000 $\times$  (Princeton Applied CR-4 preamplifier) and the maximum fundamental frequency component measured on a wave analyzer (Hecker 302A). Ten microvolts was chosen as measurement level. All experiments conducted with the animal in a sound booth (Industrial Acoustics Co. Inc.).

## RESULTS

CM sensitivity functions for the 6 albino and the group of 6 pigmented guinea pigs are plotted together in Fig. 1. They represent the mean 10  $\mu$ V isopotential for 20 frequencies for the two groups. Both have the same general shape and at low frequencies they are nearly superimposed. The albino guinea pig sensitivity function (open circles), seems to exhibit a bias towards lower sensitivity compared to the pigmented guinea pig sensitivity curve (closed circles). For most frequencies the means of the two groups lie within the ranges of the other. Table I

A non parametric statistic, the Mann-Whitney U test, was used to test for the significant difference between the means of the two

Table II For each of 20 frequencies, the probability (Pr) of the occurrence of the experiment ( $\mu_1$  and  $\mu_2$ ) are the same as determined by a two-tailed Mann-Whitney U test

Frequency (kHz)	0.07	0.1	0.2	0.3	0.4	0.5	0.6
Pr( $H_0: \mu_1 = \mu_2$ )	0.396	0.818	1.00		0.005	0	0.333

05

	0.9	1.0	1.5	2.0	2.5	3.0	3.5	4.0	5.0	6.0	7.0
0	-156	-175	190	-150	-105	-50	-37	-79	-47	-80	120
5	-210	224	-210	-192	-151	-116	-134	157	163	-170	174
3	-195	-189	193	-189	-136	50	-73	101	-101	-111	142
7	253	-304	270	-213	193	-169	-149	-220	200	185	199

each frequency Table II contains the results of this test. For 15 frequencies, the null hypothesis, that the albino and pigmented groups have the same mean sensitivity, cannot be rejected at the 5% significance level. Only 5 of 0 differences (at frequencies 400, 500, 1 500, 3 and 7 000) are found significant at the 5% level.

The second measure of auditory performance reported here is the maximum magnitude of the CM. For each group of guinea pigs, Table II shows the mean of the maximum potential as well as the range of the data at 3 frequencies (1 000 and 4 000 Hz). Results of the Mann-Whitney U test on these maximums are also given in Table III. In all cases the null hypothesis cannot be rejected at the 5% significance level.

## DISCUSSION

The data indicate that, for the most part, albino and pigmented guinea pigs are alike in terms of their 10  $\mu$ V CM sensitivity functions and the maximum CM magnitude which can be generated. Bias in the direction of less sensitivity in the albino data may well be due to one animal which performed poorly. Indeed, 10 of 15 frequencies from this animal represent the low sensitivity end of the group range. At 500 Hz where the separation between the groups is greatest, the difference between the group means is 5.6 dB. This value does

not seem large when compared to the standard deviation, at 500 Hz, of 10 dB obtained by Lawrence & Yantis (1957) for 35 normal guinea pigs. In that study, Lawrence and Yantis report standard deviations on a 1  $\mu$ V sensitivity function which range from a minimum of about 7 dB to a maximum of about 16 dB. However, it should be noted that a 10  $\mu$ V CM sensitivity level may have smaller variance than the 1  $\mu$ V CM level because of a greater signal to noise ratio, i.e., relatively less uncorrelated physiological noise and possibly less contamination by acoustic nerve action potentials.<sup>1</sup>

The above findings may be compared to the available behavioral data. Crifo (1973) reports that the 2 guinea pig groups are quite different in terms of their auditory thresholds. He observed a 23.3 to 33 dB difference between the animal strains with the pigmented animal being less sensitive (recall that the possible bias seen in the CM data suggests that the pigmented guinea pig is more sensitive). All frequencies which Crifo tested were significant at the 0.1% level.

Relatively little research has been done concerning the relationship between the CM and the hearing threshold. Wever (1959) has compiled correlatable behavioral CM data on five species, including the guinea pig, and concluded that the general shape of the CM and behavioral

<sup>1</sup> Weiss et al. (1971) reported intensity nonlinearity possibly caused by neural contamination.

given the null hypothesis ( $H_0$ ) that the pigmented and albino guinea pig mean 10  $\mu$ V sensitivities

0.9	1.0	1.5	2.0	2.5	3.0	3.5	4.0	5.0	6.0	7.0
0.064	0.240	0.042	0.004	0.180	0.240	0.438	0.180	0.132	0.396	0.042

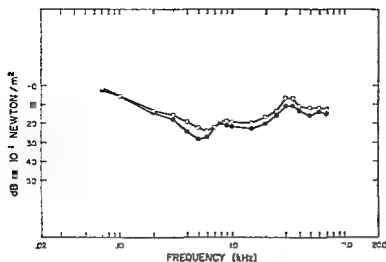


Fig. 1 Mean sensitivity (10  $\mu$ N) curves for albino (—) and pigmented (—●—) guinea pigs.

functions for a given species differ in three aspects, the low frequency slope, the point of maximum sensitivity, and the upper limit of response. Additional reports have expanded the number of species tested and have confirmed an interspecies variability in both functions (e.g., McGill, 1959; Dalland et al., 1967; Price, 1971). Part of the difference between the CM and behavioral functions can be accounted for by the very nature of the CM signal recording technique. An electrode placed near the inner ear will tend to favor signals from the nearby sources of the spatial array. Thus, the round window recording technique shows bias for low audio frequency signals since the receptor of the first cochlear turn are closest to the

electrode. Discordance between the behavioral functions may also be from measurements of the behavioral field (e.g., closed or open field). When considering the above points it is not surprising that the shapes of the CM and behavioral threshold functions are not alike.

There is reason to believe that the shape of either the CM or the behavioral threshold function, induced by ear manipulation, may be a useful measure of ear function. Changes in CM after overstimulation have been correlated with ear structural damage seen histologically (et al., 1944; Davis, 1953). Additionally, hearing loss which results from acoustic or ototoxic drugs also correlates with these findings (Suggit, 1943; Elliott & McGee, Stebbins et al., 1969). Experiments which combine induced changes in CM with behaviorally determined auditory thresholds and histology are lacking at this time. These findings imply the possibility that a behavioral difference in behavioral auditory threshold could also be manifested by the microphonic potential.

In conclusion, this study indicates there is little or no difference in the CM functions and maximum CM magnitude between pigmented and albino guinea pigs. These findings therefore, do not appear to support the conclusions of Cniffo (1973) that the two species

Table III The mean and range of the greatest CM produced by a group of 6 albino and a group of 6 pigmented guinea pigs

"Pr{ }"—The probability of the occurrence of the experimental samples given the null hypothesis ( $H_0$ ) that the pigmented and albino guinea pig mean maximum CM voltages ( $\mu_1$  and  $\mu_2$ ) are the same as determined by a two-tailed Mann-Whitney  $U$  test.

Frequency (kHz)		0.01	1.0	4.0
Pigmented CM ( $\mu$ V)	Maximum	1100	970	580
	Mean	875	785	456
	Minimum	700	600	350
Albino CM ( $\mu$ V)	Maximum	1100	890	500
	Mean	667	715	382
	Minimum	450	490	260
Pr( $H_0: \mu_1 = \mu_2$ )		0.31	0.588	0.094

cantly different hearing thresholds. However, one must remain aware that electrophysiological and behavioral methods are not necessarily equivalent measures of auditory function. It is obtained by each of the two methods conceivably reflect entirely different aspects of the hearing process since, as mentioned, the two methods have not been experimentally compared.

## ACKNOWLEDGEMENT

The author wishes to thank Mr David Marques for his assistance in collecting some of the data.

## ZUSAMMENFASSUNG

Verhaltensteste scheinen darauf hinzudeuten, dass die Hörschwelle von Albino-Meerschweinchen signifikant höher ist als bei pigmentierten Meerschweinchen. In vorliegenden Untersuchungen wurden cochleäre mikroelektrische 10  $\mu$ V Isopotentiale ("CM Empfindlichkeit") im Frequenzfenster dieser 2 Gruppen am anesthetisierten Meerschweinchen (*Cavia procavia*) als ein Mass der Hörleistung gemessen. Bei 15 von 20 untersuchten Tieren (zwischen 70 und 7 000 Hz) war die durchschnittliche CM Empfindlichkeit von 6 Albino-Tieren mit durchschnittlicher CM Empfindlichkeit der 6 pigmentierten Tiere nicht signifikant verschieden. Die maximal erreichbare cochleäre Potentialhöhe bei 3 Frequenzen (100, 1 000 und 4 000 Hz) bei den Gruppen war statistisch ähnlich. Wir schliessen daraus, dass ein Unterschied zwischen den untersuchten Meerschweinchenstämmen hinsichtlich der gemessenen Parameter des cochleären mikrophonischen Potentials besteht.

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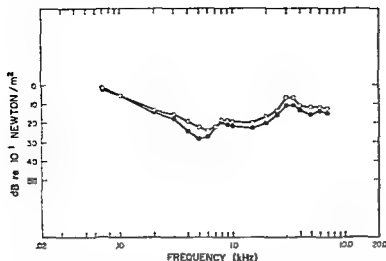


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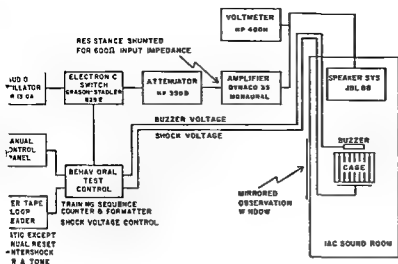


Fig 1 Diagram of testing apparatus

exposure The exposure itself is more open question. We have tried putting the sound directly to the tympanic membrane through a drum. This makes it possible to measure the intensity of the stimulus with a probe tube from microphone inside the orifice of the speculum. A discrepancy may arise in that the animal, when anesthetized and clamped as securely as possible, may still achieve slight movements. A slight movement may move the speculum to a slightly different position and so a quite different intensity of sound may be delivered to the tympanic membrane. We have also tried delivering the sound from a speaker three inches from the pinna, and aimed directly at the external orifice of the external auditory meatus, with a probe tube just inside the orifice. This gives an instant sound level, but we do not know exactly what the sound level is at the drum membrane. This seems to be the most consistent way we can deliver the stimulus, therefore, despite the doubt as to what the level is at the tympanic membrane, it seems better to achieve consistency where it can be measured accurately.

## MATERIALS AND METHODS

The study was conducted along classical lines. That is, the work can be divided into the following separate parts. Behavioral training

and determination of audiograms, exposure to pure tones, redetermination of the audiograms, sacrifice, and examination of the organ of Corti. These represent distinctly separate disciplines which should be managed by experts in each area, thus the multiple authorship of this paper. Dr Ades was in overall charge of the project and also participated especially in the anatomical work, Dr Kokko-Cunningham took part in the anatomical portion and had the special responsibility for the electron microscopy and histochemistry, Dr Trahiotis supervised the animal testing portion, and Dr Averbuch, who is an electrical engineer, supervised the exposure and the instrumentation.

## PROCEDURES

### Apparatus

Five monaural chinchillas were trained in the apparatus which is diagrammed in Fig 1.

The output from the audio oscillator was delivered to the electronic switch for gating the tone on and off. The gated tone was adjusted in amplitude with an attenuator from a known value of speaker drive (measurements taken with a voltmeter for a continuous tone).

The behavioral test apparatus, which was built in this laboratory, contained the electronics necessary to program the sequences of stimuli and to operate the buzzer and shock device. The

# COMPARISON OF HEARING IN THE CHINCHILLA

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*Abstract* Behavioral, anatomical and histological effects of acoustic overstimulation produced in chinchillas were investigated. In each of five months, high frequency hearing loss was produced and was related to locus and extent of hair cell damage. Audiograms were fairly consistent with the behavioral data and the anatomical data on presence of hair cells. Histochemical and electron micrographs of selected portions of the cochlea revealed a series of changes in hair cells which may not influence their functional properties.

Tones or noise of high intensity have been used for many years to stimulate the ears of experimental animals and thus to produce damage to ear structures and changes in function of the inner ear. Periodically, developments in techniques for examining inner ear function have been reported.

As techniques for measuring inner ear function have improved, the closer and closer correlation has been found between the physical characteristics of the stimulus and the ear pathology and inner ear function.

The best of the older studies employed behavioral responses by inner ear elements or methods to obtain measures of auditory function. They related inner ear function with parameters of acoustic input, frequency, and duration of sounds used. However, in very few of these studies has damage and ear function been studied in the same animal after controlled exposure.

The present study attempts to correlate three components as well as the physical

This study was carried out under grants from the Aeronautics and Space Administration (N00014-005-074) and National Institutes of Health (Number 1 R01 NS09983-01).

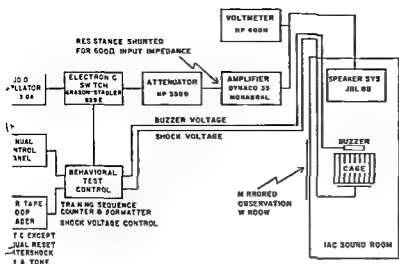


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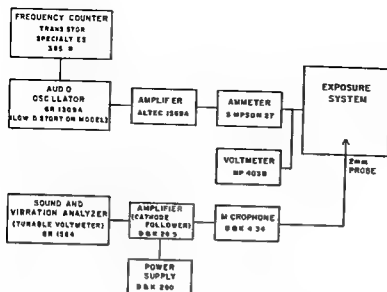


Fig. 2. Diagram of experimental setup.

training sequences could be manually triggered from the control panel, or could be sequenced automatically by a paper-tape loop. The paper tapes were computer generated and provided the desired randomization of intertrial interval.

The sound pressure level (SPL) for a known value of speaker input voltage at one frequency was measured for all frequencies at 24 points in the training cage. The reference SPL used at any frequency for threshold determinations was the average of these 24 readings. Calibration in this manner allowed all SPL's at all frequencies to be known with respect to a single, pre-set driving level without the need for readjustment.

#### Audiometry

The chinchillas were trained to avoid shock by crossing a barrier in the shuttle box (see Fig. 1) in response to presentation of a 2-sec sample of a sinusoidal tone of 1000 Hz. Shock was accompanied by sound from a buzzer mounted in the roof of the cage. Thus, the sound of the buzzer acquired aversive value and could substitute for the shocks to keep the animals motivated in the months of future testing.

When the animals had reached a criterion of not less than 90% correct avoidance responses, the stimulus parameters were changed, and threshold determinations were begun using a method of descending limits. The duration of

test tones was 750 msec with a rise time of 50 msec. The stimulus consisted of bursts separated by 250 msec. If the animal moved from one end to the other of the box at any time during the presentation of three tone bursts, shock was not delivered. If a correct response was recorded, the stimulus was then attenuated by 10 dB. The test was repeated after a random time interval (averaging about 20 sec). The test was repeated until the animal responded. The SPL was then increased until the animal responded correctly, the stimulus was reduced 10 dB and the sequence continued. If the animal failed to respond to the stimulus, the increase in SPL, the procedure was discontinued and the threshold was taken to be the level between the latter level and the level of the last positive response. Each animal was tested at 2, 4, 8, and 16 kHz, or 0.1, 0.5, 1.0, 5.0 and 10 kHz each day. Since the animals got better and better at detecting low frequencies, many measurements of "threshold" had to be taken. When these remained stable over at least 20 sessions at each frequency, a threshold value was calculated.

Following completion of the tests, the animals were tested at three levels of noise using a low pass noise filtered at 10 kHz. The noise was presented continuously at room

of 0 dB, 10 dB, and 20 dB SPL. Measurements of levels of noise were made at 24 positions in the test cage. The sound field was quite uniform. Each animal was tested at least eight times at each test frequency for each of the three levels of masking noise.

#### *Exposure to pure tones*

Each of the animals was anesthetized and exposed to presentations of a 4 kHz tone for one hour at 130 dB, 125 dB, or 120 dB SPL. A block diagram of the apparatus is shown in Fig. 2. A specially constructed plastic chamber was used for exposing chinchillas to sound levels of 140–145 dB SPL. The maximum attainable sound levels were dependent upon frequency, due to resonances in the rectangular exposure chamber. The basic chamber was made of one-half inch thick transparent plastic, 4 inches high, 4 inches wide, and 13 inches long internally and open at the ends. On one end, any of a variety of speaker fittings could be attached. An Altec 421A, 15-inch, low frequency speaker could be mounted, with an adapter, for frequencies below 500 Hz. For the lower frequencies, heavy aluminum extensions could be added to tune the length of the chamber to the desired resonant frequency while the end of the chamber, opposite the speaker, was sealed with a thin aluminum plate. For higher frequencies, a pair of Altec 290 D speaker drivers were connected to the chamber through a cast aluminum horn. The speakers were driven in phase. When necessary to avoid chamber resonance, an acoustic absorbing material was attached on the end of the exposure chamber opposite the speaker. With the animal's head in the chamber, a 2 mm probe was inserted into the ear of interest, and was brought as near as possible to the ear canal opening. The probe, attached and calibrated to a one half inch microphone, was left in place during the entire exposure. In addition to the SPL of the principle exposure frequency, the first few harmonics and subharmonics were monitored with a tunable voltmeter to insure against excessive distortion. The absolute sound level calibration was made with a B&K 4220

"Pistonphone" coupled to the one half inch microphone. The probe was calibrated, over the frequency range of interest, against a second one-half inch microphone.

#### *Anatomical investigation*

The surface preparation used for the initial phases of anatomical study was described in Stockwell et al. (1969). It will not be repeated here. The following histochemical methods were employed.

The cochleas (with the exception of animal No. 89) were fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer of pH 7.2. The fixation was begun by diffusing the fixative gently through the fenestra ovalis and the fenestra rotundum, with a small outlet hole made in the apex. It was continued by immersing in the same fixative for 24 hours. During the latter period the bony walls of the cochlea were ground down and the coils of the organ of Corti were detached and removed from the modiolus. The resultant segments were treated as free floating pieces thereafter. When fixation had been completed, the segments were rinsed from 12 to 24 hours in 0.1 M cacodylate buffer (pH 7.2) which contained 0.2 M sucrose. They were then incubated for acid phosphatase activity as described by the Barka-Anderson (1962) modification of the Gomori reaction (1952). Incubation time was 60 min at 37°C, after which the segments of the organ of Corti were rinsed again and then placed in 1% OsO<sub>4</sub> in cacodylate buffer for 1 hour. Following another rinse in the buffer, the segments were mounted in glycerine under cover glasses. They were then ready to be observed under the light microscope. Animal No. 89 was fixed directly in 1% OsO<sub>4</sub> buffered with veronal acetate and no incubation was done.

After recording the presence, absence, or damage of hair cells of the organ of Corti (the resultant computer drawn cochleogram will be illustrated for each animal), the segments were removed from the slides, dehydrated, embedded in Epon-Araldite, and tissue from representative areas was thin sectioned. In some specimens, only a narrow area was sampled for elec-

iron microscopy, while in others segments up to 1000  $\mu\text{m}$  in length were sampled at 25 to 50  $\mu\text{m}$  intervals. Thin sections were stained with uranyl acetate and lead citrate and viewed in either the RCA EMU3H or the Siemens Elmiskop A1.

## RESULTS

### Pre-exposure data

The mean thresholds of the 5 animals taken in quiet circumstances are shown in Fig 3 in solid line. In addition, the similar curve for Miller's 36 animals is shown in broken line (Miller, 1970). This demonstrates a comparison with the results of at least one other investigator, and shows general agreement with work done on the same species at a different laboratory, with different apparatus. The two differ significantly only at the highest frequencies, 8 kHz and 16 kHz. This may be the result of some slight difference in the test chambers which is impossible to analyze.

An indication of the variability of threshold measurements can be gained from Fig 4, in which representative data from 2 animals, Numbers 90 and 96, are plotted as a function of number of thresholds included in the determination of average threshold. Standard deviation was constant at about 5–10 dB in the last five measurements. Fig 5 shows the average thresh-

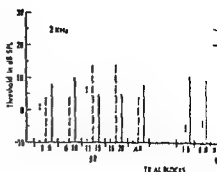


Fig 4 Samples of mean, median, and standard deviation of pre-exposure threshold responses of animals 90 and 96 in blocks of five trials.

olds of the 5 animals for four conditions of pre-exposure testing, i.e., quiet and 0, 20 dB levels of masking. No data were available at 16 kHz in the presence of masking because of the variability in the sound level caused by the non-uniform speaker response. The non-uniform speaker response was excluded by adequate definition of the level of the masking. The thresholds were systematically at the level of the masking. The apparent insensitivity of the animals at 1 kHz is the fact that the sound system had a null point at that frequency.

It is possible to compare the signal-to-masker level ratios necessary for detection at each test frequency to the data reported by Miller (1970, p. 176) (from Miller J., unpublished observations). In order to make these com-

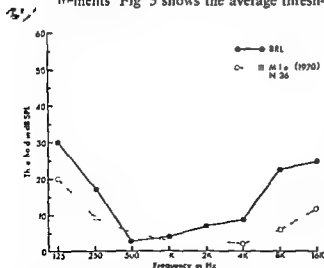


Fig 3 Average pre-exposure thresholds of the 5 animals included in this study (—) and of Miller's 36 animals done at Central Institute for the Deaf (---).

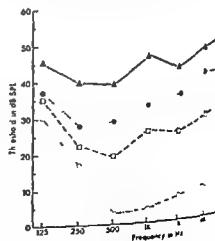
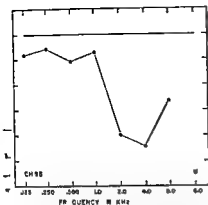
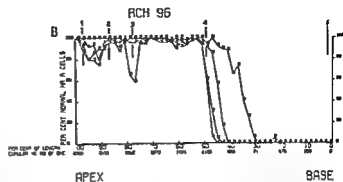


Fig 5 Pre-exposure thresholds in quiet (—) and three levels of masking (--- = 0 dB level, - - - = 10 dB level, . . . = 20 dB level).



(A) Hearing loss animal No. 96 following exposure to 4 kHz, 130 dB for 1 hour (B) Cochleogram of animal No. 96 (1 line—inner hair cells; 2, 3 lines—



rows of outer hair cells from modiolus side out). Vertical lines and numbers indicating where samples were taken for EM

actual SPL value of the noise spectrum level used in the test cage was utilized. The signal spectrum level ratios measured in the two studies typically do not differ by more than 2 dB. The test frequencies used in both studies result is interpreted as further evidence of frequency of the sample and the testing procedures.

#### Exposure data

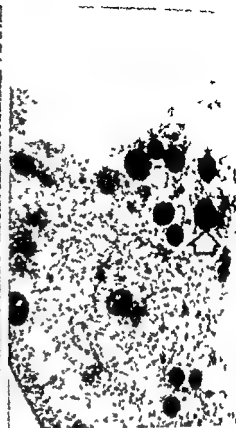
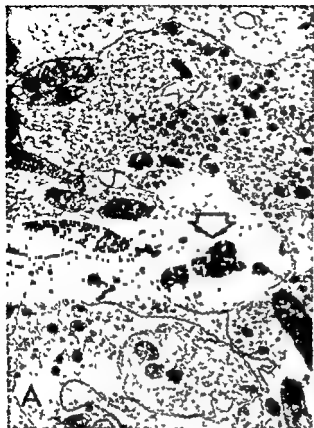
Animal No. 96 Fig. 6 is a composite of the data for this animal. Fig. 6A is the curve of hearing threshold with normal hearing assumed to be a straight line as is ordinarily done with human data in audiogram. Loss of hearing for the several frequencies is represented as points below the normal line. There is negligible hearing loss at the first four frequencies; however, there are losses of 40 dB at 2 kHz, 44 dB at 4 kHz, and 27 dB at 8 kHz. Ostensibly, the loss at 4 kHz, but it will be remembered that standard deviation is roughly 5 dB, so a 10 dB loss is probably significant but must be considered as marginally so.

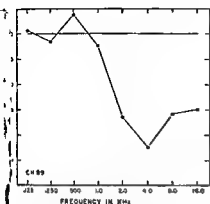
The computer drawn cochleogram (Fig. 6B) shows complete cell destruction from the base to 30% of the length, rising sharply from that point indicating a virtually intact cochlea for the remainder of the length. The inner hair cells appear to be about 75% of them being present at 35% of the length and 90% or higher from 40 to 50% of the length. There is little difference between the rows of outer hair cells in this respect. There is scattered cell loss at the apical end with only the third row of outer hair cells showing appreciable loss. Samples were taken from the specimen for electron microscopy from five areas. The areas are marked by vertical lines in Fig. 6B.

In the first three areas in addition to the missing cells, other changes that were considered abnormal were seen. Some fibers in the inner spiral bundle and nerve endings underneath the inner hair cells appeared swollen (Fig. 7A). Outer hair cells in all three areas showed an accumulation of inclusion or multivesicular bodies under the cuticular plate (Fig. 7B). These vesicles were surrounded by membranes and contained granular or fibrillar material and a few lipid droplets. They varied in size from 0.5 to 2  $\mu$ m. The inclusion bodies occurred in moderately increased number as compared with the normal animals, and some of them exhibited acid phosphatase activity. Lamellar structures, some what resembling tightly packed lateral membranes were also seen under the cuticular plate in the outer hair cells of these areas.

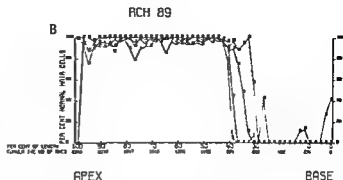
In area 4 many more of the multivesicular bodies appeared in the outer hair cells, however, the region in which the cells contained abnormal numbers of these was relatively narrow.

Area 5





(A) Hearing loss animal No. 89, following exposure to 4 kHz, 125 dB for 1 hour (B) Cochleogram animal No. 89 (see legend for Fig. 6B)



only 200  $\mu$ m from the area of heavy damage is in contrast to animal No. 93 (see below). It had a wider area of abnormal outer hair near the edge of heavy damage. In addition, fibers of the inner spiral bundle were swollen, and occasionally fused, or giant stereocilia on both inner and outer hair cells were present. Rods or crystalloids exhibiting a tubular structure were more numerous than those usually seen in inner hair cells of normal animals. They showed signs of degeneration in that they contained occasional lipid droplets (Fig. 7C), and sometimes showed acid phosphatase ac-

cumulation. At a distance of 5, 400  $\mu$ m from the base of the cochlea, there was only a flat epithelium. Cytoplasmic granules were scarce. The cells of the limbus were fewer than normal and quite swollen while the Böttcher cells appeared normal.

**Animal No. 89** This animal showed a hearing loss curve (Fig. 8A) much like the preceding one, with loss peaking at 4 kHz. The peak is

at -45 dB. The only other frequencies which show significant losses are 2, 8, and 16 kHz.

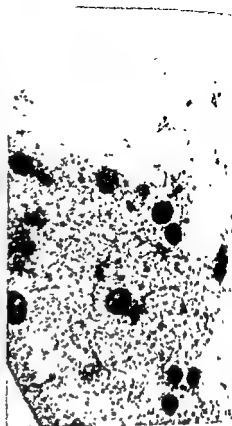
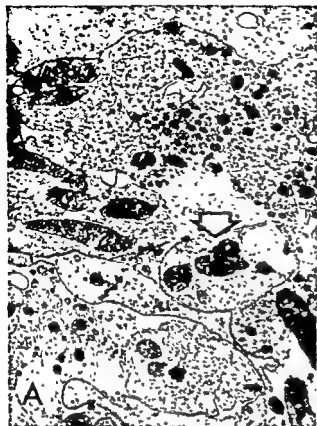
The cochleogram (Fig. 8B) shows essentially a complete absence of hair cells (except for a minor number of inner hair cells) up to 30% from the basal end of the organ of Corti. This is a fairly sharp-edged lesion with the inner hair cells again virtually completely present within 2%, i.e., at about 32% of the length. The outer hair cells are all present once more at about 43% of the length. From that point on to the apex there is scattered, minor loss of outer cells and the inners remain at full count.

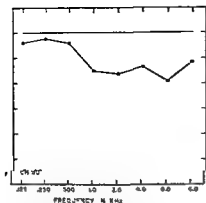
Electron microscopy was done, but the ultrastructural preservation was inferior to the other 4 animals. Consequently, about all that can be said affirmatively is that there was an abnormal accumulation of multivesiculated bodies in the outer hair cells near the major lesion. Systematic sampling was not carried out.

**Animal No. 97** This animal showed distinct loss of hearing at 0.250, 0.500, and 1.0 kHz, and a much greater loss at 2, 4, and 8 kHz, the

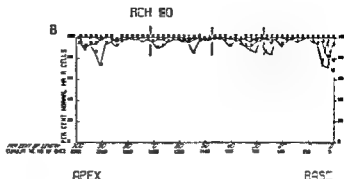
(A) Inner spiral bundle from area 1 (middle of middle coil) of animal No. 97. Some of the nerve fibers are swollen (arrow) and have empty spaces. Others are normal and filled with fibrillar material. 11 000 (B) Outer cell 2 from area 4 (end of middle coil) of animal No. 97 showing accumulation of multivesicular inclusion bodies (arrow points to one) under the cuticular plate. The bodies are surrounded by a membrane and contain fibrillar or granular material and lipid like droplets. 11 700 (C) Inner hair cell from area 4 (end of middle coil) of animal No. 96. One of the rods with tubular sub-structure shows accumulation of lipid (arrow). 6 300 (D) Outer hair cell 1 from area 6 (lower basal coil) of animal No. 93. Multivesicular inclusion bodies have increased in number and fill the subcuticular cytoplasm. Some have lost their surrounding membranes and small lipid like droplets are dispersed in the cytoplasm. 11 200

lets. 11 700 (C) Inner hair cell from area 4 (end of middle coil) of animal No. 96. One of the rods with tubular sub-structure shows accumulation of lipid (arrow). 6 300 (D) Outer hair cell 1 from area 6 (lower basal coil) of animal No. 93. Multivesicular inclusion bodies have increased in number and fill the subcuticular cytoplasm. Some have lost their surrounding membranes and small lipid like droplets are dispersed in the cytoplasm. 11 200





1 (A) Hearing loss, animal No 90, following exposure to 4 kHz, 120 dB, for 1 hour (B) Cochleogram of 1 No 90 (see legend for Fig. 6B)



• lower three frequencies, 0.125, 0.250, and 0.5 kHz (Fig 10A). This statement is based on the thesis that nothing below a 10 dB loss could be counted as an actual loss.

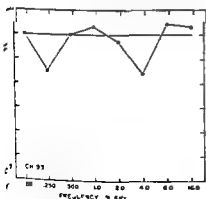
The cochleogram (Fig 10B) shows little damage. The greatest amount is just at the base, up to 10% of the length, the loss being about

in the three rows of outer hair cells. The order of the length shows only significant deviations of one of the rows at a time, one deviation near the apex showing 25% go to the third row of outer hair cells. Inner hair cell row is intact throughout. Sites for electron microscopy were taken in the areas indicated in Fig 10B.

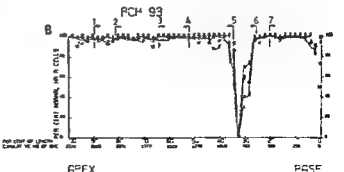
Part from the missing cells only a few ultrastructural changes were interpreted as abnormal.

Outer hair cells in all areas sampled showed an accumulation of inclusion or multivesicular bodies under the cuticular plate. There were occasional giant stereocilia in all the samples, more frequently on inner than on outer hair cells. The nerve endings and supporting cells appeared normal.

**Animal No 93** This animal had apparently significant losses of hearing at 0.250 and 4 kHz, and normal hearing otherwise (Fig 11A). The loss at 4 kHz would fit the results on other animals, at least it would not be discordant. The loss at 0.250 kHz, however, fails to match the results on the others. In no other case are there losses at two test frequencies with an intervening interval of three test frequencies at which no loss occurs. Whether this is to be "explained" on



1 (A) Hearing loss, animal No 93, following exposure to 4 kHz, 125 dB, for 1 hour (B) Cochleogram of 1 No 93 (see legend for Fig. 6B)





the basis of some unknown error in testing or on the microscopic results remains to be seen. The cochleogram (Fig 11B) shows a complete loss of cells over a short distance at about 30–39% of the length counting from the base. This might account for the 4 kHz loss in hearing but there is no separate lesion to account for the loss at 0.250 kHz. The two hearing losses are of about equal magnitude. If the 4 kHz loss is to be accounted for as being all that would be expected from a narrow lesion in the basal turn, why is there no obvious lesion in the apical turn to account for the loss at 0.250 kHz? On the other hand, if the result on 0.250 kHz is to be accounted for on the basis of testing error, why is that at 4 kHz not equally spurious? The specimen was sampled at seven regions shown by vertical lines in Fig 11B.

The first four areas (counting from the apex) all showed swollen nerve endings under the inner hair cells and swollen fibers in the inner spiral bundle. Outer hair cells showed an increase in the number of inclusion bodies under the cuticular plate (Fig 7B). Rods or crystalloids exhibiting degenerative changes were seen in inner hair cells, however, we are not prepared to say that the changes noted are related to the hearing loss at 250 Hz.

In areas 5 and 6 the main lesion consisted of a central area about 250  $\mu\text{m}$  in width in which the acoustic papilla was absent and was represented only by flat cuboidal epithelium which was relatively empty of cellular organelles. The hair cells were missing in an area about 250  $\mu\text{m}$  wide in either direction but in these zones the supporting cells were present filling the space formerly occupied by hair cells, and maintaining the general contour of the organ of Corti. There were acid phosphatase positive lysosomes and Golgi complexes in the supporting cells. Cell debris from degenerated hair cells was rarely seen, and in these areas there were numerous nerve fibers which were quite normal in appearance. In the region from 750–1000  $\mu\text{m}$  away from the maximum damage area the subcuticular portions of the outer hair cells were filled with multivesicular bodies (Fig 7D) which con-

tained large numbers of lipid inclusions. The area from 500–750  $\mu\text{m}$  from the damage were outer hair cells in which the vesicular bodies not only occupied the subcuticular region, but filled the entire cell. The multivesicular bodies had lost their surrounding membranes and their contents consisting of lipid droplets were dispersed out the cytoplasm of the hair cells. The numerous degenerated nerve endings in the outer hair cells in the area also. The inner cells contained many tubular rods and showing degenerative changes. Giant nuclei were relatively frequent.

In area 7, the outer hair cells still less than the normal complement of multivesicular bodies in the subcuticular region. Under the hair cells there were many swollen nerve endings. Inner hair cells also contained degenerated with occasional acid phosphatase positive numerous giant stereocilia were seen.

## DISCUSSION

Many articles on the correlation between hearing loss and damage to parts of the ear start with at least an implicit assumption that the place of maximum vibration of the membrane determines receptor response to acoustic stimuli. By a curious coincidence this 'place' theory of hearing then explains the data while the data provide support for a 'place' theory of hearing. It is as if one looks only at 4 kHz exposures of the ear with a simple place theory in mind but by no means perfect. The present study is only to exposures of 4 kHz and is somewhat biased toward belief in a place theory. It should not be so considered and the place arguments should be held in abeyance at the moment. This paper is designed to relate to the problem of correlation of damage in the inner ear and damage to function.

In that respect there are some discrepancies between hearing results and pathology in animals. Nos. 89 & 90 are according to a rigorous place theory.

loss at 4 kHz should be much higher than it is not complete. Likewise, hearing for the higher frequencies should be as much impaired at 4 kHz if not more, inasmuch as the receptors were destroyed for the more basal portions of the cochlea as well as the presumptive 4 kHz region. In actual fact, however, a considerable amount of hearing remains for those frequencies. The receptors are transducing the higher frequencies.

Before attempting an answer to that question, we turn to the histological methods for a comment. A source of error may be found in the manner of judging the condition of the cells from the construction of the cochleogram alone. This is the object of electron microscopical and chemical investigation of the same specimens as are represented in the cochleograms will form the subject of a separate publication, but some comment is warranted here. In the immediate vicinity of the lesion as is shown on the cochleograms show varying degrees of abnormality including ultrastructural and histochemical changes. It must be said the ultrastructural preservation left much to be desired. It was originally thought necessary to mount the specimens in glycerine to count on for the construction of cochleograms. We attribute part of the inferior preservation to this addition, the long rinses and incubation in an osmium tetroxide solution after fixation also contribute to poor preservation. Even so, the changes interpreted as pathological in the present material were not found in normal animals treated similarly.

Most of the changes which we interpreted as abnormal have been described earlier. The swelling of afferent nerve endings and fibers of the inner hair cells after acoustic trauma has been reported by Spoendlin (1971). He described it as a reversible change. Spoendlin (1970) reported similar swellings as a result of changes in osmotic pressure (like the fused stereocilia, but this change is not specific to acoustic trauma but is a general reaction to more than one kind of noxious influence (Engström et al., 1966)). The survival time for our animals was sufficiently long to allow for the disappearance

of all reversible changes, and, unlike Spoendlin, we interpret them as permanent, however, the number of swollen endings in our specimens apparently is less than those described by Spoendlin (1971), and might be presumed to be the irreversible residue of a larger number.

Increase of lysosomes in the infracuticular region has been reported by other authors (e.g., Engström et al., 1970; Ishii et al., 1968) after both acoustic trauma and treatment with ototoxic antibiotics. Ishii et al. (1967) also reported an increase in lipofuscin pigment and acid phosphatase activity in the hair cells of elderly humans. The inclusion bodies somewhat resemble lipofuscin, however, they usually have numerous small rather than a single large lipid droplet. The disappearance of the surrounding membrane, and the release of the lipid droplets into the cytoplasm have been illustrated by Spoendlin also (1970).

With survival time of 1-2 hours, Hensen bodies have been seen to increase in number and complexity after exposure to jet-engine noise (Engström & Ades, 1960). Several Hensen bodies were encountered in the hair cells of the present material, though no more than in normal ears. The accumulation of membranous material, similar to the lateral membranes, which was particularly prominent in animal No. 96, was also described by Spoendlin (1970) after acoustic trauma.

Some of the changes are designated "degenerative." These are degenerative changes on general grounds. That is, they are the sort of changes that would indicate degeneration in any cell. But how far does degeneration have to go to render the cell non-functional, or semi-functional, or functional intermittently? What does this do to threshold measurements? No answers are available immediately. We are dealing with uncertainties and variables at both ends of the process, and no method is yet known which will resolve the difficulty.

Perhaps the thresholds obtained in the presence of masking noise following the creation of cochlear lesions may help to explain the small amounts of hearing loss reported. One might

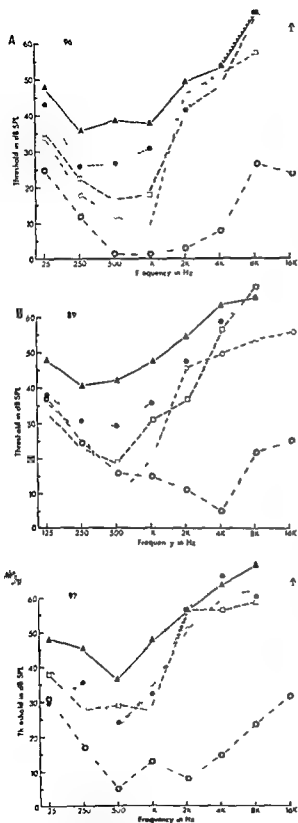


Fig 12 (A) Animal No. 96. Curves showing thresholds obtained pre-exposure (solid circles with stars), post-exposure thresholds obtained in quiet (○) with 0 dB level masker (○·) with 10 dB masker (●) and with 20

justifiably wonder how animals 96 and 97, with some of the basilar membrane stripped of receptors, hear so well. This is indicated by data in Figs. 12A and 12B. Introduction of masking noise affects performance not only at the lower frequencies (8 kHz). These data may be interpreted as indicating that the animals were using receptors in the middle regions of the cochlea to transduce 8 kHz information. Note the presence of microscopically healthy tissue in the middle regions of the cochlea and the attendant effects (Figs 6B and 8B).

Animal No. 97's data allow for a different interpretation. Note that animal 97 differed from 96 and No. 96 in the number of inner hair cells present in the basal region of the cochlea and a greater number of hair cells missing on the apical side of the cochlea. One may speculate that the inner hair cells were mediating the behavioral responses on this animal (Fig. 12C). Further, we predict that the masker effectiveness is reduced at the lower test frequencies (0.5, 1.0 kHz) because of the cochlear losses in the region 50–80% of the distance from the base. Since the animal could not efficiently transduce the lower level frequencies, the noises could not mask. Indeed, the data indicate that this is the case. When the masker was sufficient to mask the lower frequencies, a masking effect also appears to be a masking effect. Whether this indicates that the 8 kHz was processed by the animal primarily in the middle portions of the cochlea by inner hair cells or in the basal regions by outer hair cells is a difficult question to answer. The data could be interpreted as indicating that inner hair cells were transducing the information at 8 kHz and were finally masked at the noise level. The interpretation is based on the assumption that the outer hair cells were to about 80% of the distance from the base.

dB masker (A) (B) Animal No. 97 (see legend for Fig. 12A) (C) Animal No. 98 (see legend for Fig. 12A)

ed, and, if used, would result in a greater loss than that measured in the quiet. The results on masking may actually provide support for the anatomical findings. That is to say, the remaining inner hair cells in animal No. 96, and the remaining outer hair cells in animal No. 96, may be functional in whole or in part despite the evidence of changes in them. These changes, from one point of view, may be considered to represent primarily metabolic changes. In turn, this would tend to vindicate the surface reaction and, hence, the cochleograms. The results of the present experiments are at variance with those reported by Ward & Duvall (1972). There seems to be some doubt about the validity of that series of experiments (personal communication). There is also doubt that their histochemical analysis went far enough. At least, we have never been convinced that positive results were forthcoming from the type of methods that were used. Similarly, these results have no special bearing on those of Spoendlin (1972), who reported that 95% of the fibers emanating from the spiral ganglion of Corti innervate inner hair cells, and the remaining 5% innervate outer hair cells. The 5% seems a remarkably small percentage. Assuming that the outer hair cells are related functionally to loudness, however, there is no satisfactory reason to assume any exact relation between loudness detection and number of afferents innervating the receptors. Perhaps the small percentage seems small is our preoccupation with loudness in this series of experiments. This is not the precise, clear statement of facts that we would like to present. On the one hand, the masking method is open to question. On the other, the anatomical methods are imprecise. Added to this is the suspicion that the subject of the experiments, the chinchilla, is an inadequate animal on which to base broad conclusions about human hearing which is, after all, one of the objects of research of this kind. The chinchilla is simultaneously more sensitive to noise and less sensitive to frequency differences than human. Further studies should be done on

monkeys which are apparently more closely similar to humans in those respects.

## ACKNOWLEDGEMENTS

The authors would like to acknowledge the efforts of William Seaton for training the animals, Stephen Borbely for doing much of the cochlear counting, Robert Ciccone for the drawings, and Claire Ades for her editorial work. Thanks are also due to the Center for Electron Microscopy for the use of their instruments.

Professor Hans Engstrom, Head of the Department of Oto-Laryngology at Uppsala University, has consulted extensively on this project and others at the University of Illinois, and his continuing help is gratefully acknowledged.

## ZUSAMMENFASSUNG

Die Wirkungen akustischer Überreizung auf anatomische, histochemische und Verhaltens Parameter wurden an fünf einohrigen Chinchillas untersucht. Beschallung erfolgte mit einem Dauerton von 4 kHz. Die an Hand von Audiogrammen festgestellten Hörschwächen stimmten ziemlich gut mit der Lokalisation sowie der Ausdehnung des Haarzellen untergangs im Cortischen Organ überein. An Haarzellen ausserhalb der Hauptzerstörungsherde wurden ferner eine Reihe von histochemischen und ultrastrukturellen Veränderungen festgestellt, deren funktionelle Bedeutung noch unklar ist.

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## TEMPORARY THRESHOLD SHIFTS PRODUCED BY EXPOSURE TO VIBRATION, NOISE, AND VIBRATION-PLUS-NOISE

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(Received December 3, 1973)

*ct* Changes in auditory sensitivity were recorded by Bekésy audiometer technique following exposure to each of 300 cpm (5 Hz) and 1000 cpm (20 Hz), to broad band noise of 82 dB SL and to Vibration at a frequency of 300 cpm and an amplitude of 6 mm, or at a frequency of 1000 cpm and amplitude of 3 mm, were applied until the subject no longer tolerate them (a 20 min period) by a controlled vibration exciter. There was no significant change in threshold sensitivity after exposure to vibration alone. Exposure to vibration and noise simultaneously caused greater threshold shifts (TTS) and longer recovery time than exposure to noise alone. This was also true with 1000 cpm vibration. It was suggested that the effects of the combined noise and vibration be the results of some disturbances of physiological homeostasis or possible mechanical interactions with blood supply.

When a person is exposed simultaneously to multiple environmental stresses of noise, vibration and other factors Occupational deafness may be caused or aggravated by the additive effects of all environmental factors, especially vibration. However, little work has been done so far on the effect of vibration on the auditory organ. The great bulk of the literature on the effect of vibration on auditory function deals mainly with animal experiments (Popoff, 1932, Temkin, 1932, Yamamoto, 1949, Seo, 1955, Esaki, M. Saki, Y., 1958, Nomi, 1958, Nakamura, K., Jauhainen, et al., 1969). In order to avoid the effects on humans, we must use experimental animals for most studies on mechanical injury,

and some of the general conclusions drawn from animal experimentation can be applied to man. However, the abdominal system of mice in the supine position has been found to have a natural frequency between 18 and 25 Hz whereas the natural frequency of the trunk of humans is known to be about 3 Hz (Goldman & v Gierke, 1961). Humans differ from animals not only in size but in physiological and anatomical structure and psychological behavior.

Consequently, the results of animal experiments must be subjected to careful scrutiny before being applied to humans. Unfortunately, we have no information on the effect of vibration on the human ear except for the reports presented by Morita (1959) and Guignard & Coles (1965), as far as we know.

Therefore, basic studies should be devised to evaluate the effect of each environmental factor alone and of all factors together in a controlled situation. The purpose of these studies is to investigate the separate effects of noise and of vibration, and their combined effect, on human hearing.

### EXPERIMENTAL CONDITIONS AND METHODS

The experimental conditions of our study included variations in frequency, magnitude, intensity, mode of application, duration, and situation of the subject, so that the mechanical

Presented in part before the 2nd Extraordinary International Audiology Congress (Tokyo), Oct 21 1965

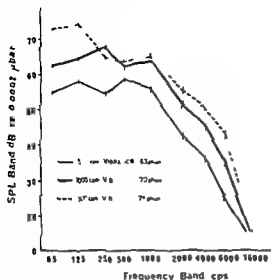


Fig. 1

responses to vibration of the body could be determined. The changes of threshold before and after these exposure conditions were evaluated by means of fixed Bekesy audiometry with a continuous tone at 4 kHz in subjects with normal hearing who had fully familiarized themselves with Bekesy audiometry.

### Subjects

Eight male members consisting of medical students and teachers in the Osaka City University took part in the test program. Their range in age was 27–35 years. They had normal hearing and had had considerable listening experience on Bekesy audiometry, i.e. they had had more than 5 practice sessions with this type of audiometry under approximately identical conditions on different days to prepare for the present experiments. Hearing of their right ears only was tested.

### Vibration exciter

The vibration exciter utilized for this experiment was the Kishida Seizo AU type vibration testing machine, which is driven by centrifugal force. It is capable of producing vertical and horizontal direction of sinusoidal vibration through a frequency range adjustable by 200 cpm up to 1 800 cpm (3.3–30 Hz). The frequency

is expressed as units of cycle per minute abbreviated cpm. The maximum stroke of the table is 5 mm, the maximum weight is 12 kg at 1 800 cpm, and the maximum weight of the test piece is 250 kg. The tests were accomplished from a console and a portable control station.

The noise produced by the vibration exciter can be a very disturbing factor in the test. The level and the frequency of the noise were controlled by enclosing the exciter with a sound proof cover, which gave the sound pressure level of below 70 dB (above 1 000 Hz) as shown in Fig. 1. The subjects also closed their ears with ear protectors during the vibration exposure. That is, since the noise produced by the device was of a low level under the present experiments and since the subjects wore earphones during vibration exposure, the effect of the vibratory device on the hearing study

### Frequency, amplitude and duration of vibration employed

Sinusoidal vibrations at frequencies of 5 and 1 000 cpm (5 Hz and 16.7 Hz) were used in the present study, because vibration at these frequencies presented the greatest motion of the body in the frequency range for humans (Cooper 1967) and the frequency distributions of random vibrations occurring in industrial environments were found to have the highest displacements in the vicinity of 1 000 cpm (Miura 1969).

All subjects were exposed to sinusoidal vibrations at each frequency, an amplitude of 5 mm at a frequency of 300 cpm and an amplitude of 30 mm at a frequency of 1 000 cpm. The subjects could not longer tolerate the vibration of 20 min. This exposure time was the longest time a subject could maintain the same posture while sitting on a shaking chair.

### Intensity level of noise employed

This was adjusted to produce a sound pressure level of 82 dB of white noise from an earphone.

, a 700 ml coupler) The earphone was  
tly fixed to the right ear

# tone and test room

pure tone ■ 4 000 Hz was selected in this  
y, as the reason that TTS producing by  
sure to white noise is the largest amount at  
Hz (Ward, 1963) The subject's thresholds  
recorded with the Bekésy audiometer (Rion,  
Model A-1014A) and an earphone (Iwasaki  
Model DR-30S, 5  $\Omega$ , ■ 8 ml coupler)  
a continuous tone at 4 000 Hz The record-  
attenuator changed attenuation at the rate  
dB/sec, in steps of 2 dB If some of the  
ects tended to confuse the threshold tone  
tinnitus or were unsure of what they heard,  
threshold stimulation tone was at once re-  
d with an interrupted tone adjusted to 10  
interruptions per sec) and a rise-decay time  
5 msec by means of the electronic switch-  
n, Model SB 02) All of the threshold meas-  
ments were carried out in a separate room  
near the vibration structure, which had  
id proof walls and was quiet enough to  
nde a satisfactory test environment

# cedure

h subject had 5 trials of series of exposures  
vibration alone, and to vibration and noise  
ultaneously The subjects were given all the  
nmental conditions in the same order and  
the same trained physician Each trial was  
ormed from 8 00 to 9 00 p.m. at intervals  
-t weeks  
efore exposure to noise or vibration, 4 min  
dy and interrupted cursive thresholds were  
ained from each subject After exposure, the  
diometric earphone was replaced, and the  
ect's threshold ■ 4 kHz was recorded as  
n as possible The post-exposure threshold  
asurements were conducted by the following  
er over 20 min duration  
o reduce any fatiguing effects due to the  
tinuous test tone, the first tracings were for  
in, 40 sec (only the threshold tracings fol-  
ing exposure to noise alone were continu-  
ly made for 4 min) and all of the subsequent

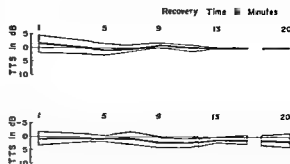


Fig 2

tracings were given with rests of 1 min alternat-  
ing with tracings for 1 min The rest before the  
final tracing of 1 min was 5 min That is, the  
recovery process of the TTS was recorded 20 sec  
(TTS<sub>0.3</sub> or initial TTS), 3 min (TTS<sub>3</sub>), 5 min  
(TTS<sub>5</sub>), 7 min (TTS<sub>7</sub>), 9 min (TTS<sub>9</sub>), 11 min  
(TTS<sub>11</sub>), 13 min (TTS<sub>13</sub>), and 20 min (TTS<sub>20</sub>)  
after stopping exposure

The cursive thresholds are represented by  
solid lines joining the midpoint of the excursion  
of the threshold tracings The identification of  
the resting threshold and these TTS values was  
made by averaging the first 10 sec periods in  
the solid line trace

# RESULTS

For the magnitude and recovery of TTSs in each  
of the exposure conditions, the mean and the  
95% confidence intervals of the TTS values  
obtained from all 7 subjects (except one with a  
pronounced auditory adaptation in the pre-  
exposure threshold tone), were calculated and  
are shown diagrammatically in Figs 2 and 3  
The resultant data are presented so that the  
open or stippled area has a width of one 95%  
confidence interval on either side of the mean

# Effects of vibration alone

The upper section (I) in Fig 2 shows the results  
obtained following exposure to vibration of 300  
cpm, and 6 mm amplitude for 20 min No shift  
in threshold sensitivity occurred in any of the  
measurements after exposure, but the first mea-  
surement after stopping the vibration showed



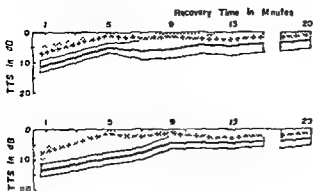


Fig 3

slight improvement in the threshold (about 3 dB lower than the resting threshold). However, the initial TTS showed considerable variation.

The TTS values produced by exposure to vibration at a frequency of 1 000 cpm and an amplitude of 3.0 mm (the lower section (2) in Fig 2) showed only a slight change in threshold sensitivity at 4 kHz for 20 min, with a small increase of TTS<sub>7</sub> to TTS<sub>20</sub>. As shown in Fig 5, some of the subjects showed evidence of a loss of about 10 dB even in post-exposure tracings for 1 min.

#### *Comparison of effect of noise alone with effect of simultaneous noise and vibration*

of the upper curves in both sections of 13 indicate the process of recovery from the elevated threshold following a 20 min exposure to a broad-band noise of 82 dB in SL. The lower curves indicate recovery following exposure to

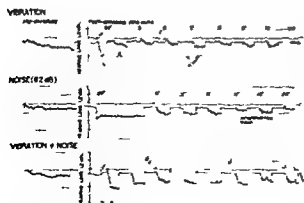


Fig 4

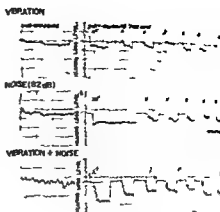


Fig 5

noise-plus-vibration of 300 cpm (upper (1)) or 1 000 cpm (lower section (2)).

The initial TTS produced by exposure alone showed a mean threshold rise and recovery to almost the pre-exposure level occurred in the short time of about 5 min.

Simultaneous exposure to vibration and noise caused the TTS to rise about a significantly greater rise than exposure to noise alone ((1) in Fig 3). Although illustrated in the figure that there are individual differences in the rate of recovery, the pre-exposure threshold, the mean curve shows a much slower recovery to noise alone. Actually the threshold was normal 5 min after exposure, then rose again at 7 min.

In many of the subjects combined TTS and delay of recovery was produced by exposure to combined vibration at 1 000 cpm and the 82 dB SL noise. The mean TTS was about 13 dB and TTS was 10 dB. The curves following noise alone and exposure to vibration were divergent for 9 min after exposure stopped ((2) in Fig 3).

Thus, exposure to a noise of 82 dB to 1 000 cpm vibration simultaneously caused auditory sensitivity more than exposure to noise plus 300 cpm vibration or exposure to vibration alone.

Although it is not shown in these figures, co-existence of noise and vibration can cause a prolongation of time to recovery.

little or no difference in the tone of the from that produced by exposure to alone. Some of the subjects showed some of the drop in short term tracings cessation of exposure to vibration alone. Early discernible change in the width of in tracings is not found when the attenuation is 1 dB/sec.

4 and 5 show the changes of acoustic to the three exposure conditions of (No 7) over a period of 20 min.

## DISCUSSION

Mechanism of the injurious effect of vibration on the acoustic organ is very little understood. From a review of the previous literature with the mechanical responses of the to a low frequency vibration (Goldman & Ke, 1961, Coermann, 1963, Miura, 1959), we attempted to elucidate the mechanism of injury caused by forced vibration. A vibratory force of a frequency of 300 cpm produces motion of the head and displacement of the thoraco-abdominal organs. Vibration does lower auditory sensitivity, it does so by both physical stimulation of the acoustic organs and physiological stimulation of the thoracic and abdominal organs, and it can be considered responsible for a disturbance in homeostasis for autonomic function.

At a frequency of 1 000 cpm, the vibration is transmitted from the excitation point to the ear so that the maximum resonance with increasing accelerative force occurs in the head and ear. The functional changes and the damage to the acoustic organ, probably including the cochlear auditory nerve tissue, may be induced by the physical factor of the vibratory force acting through the base of the skull and the jaw. Because of the anatomical peculiarity of the inner ear, with its long, narrow, complex ramifications, the disturbance following vibration probably also affects the capillaries supplying the auditory nerves, a classical example of which type of damage is Raynaud's phenomenon

produced by local vibration in many workers using such equipment as pneumatic hammers and drills.

In summary, it was suggested that the effects of the combined noise and vibration, if indeed they prove to be confirmed in future studies, might be the result of some complicated unspecified disturbances of physiological homeostasis or possible mechanical interactions with its blood supply—and let it go at that. It is not made clear whether the present findings relate in any way to the animal experiments that are cited in the introduction.

The results of these and of other experiments indicated that the simultaneous effect of vibration-plus-noise is to increase the vulnerability of the auditory organ to the effect of noise alone, and the vibration alone has a negligible effect. However, according to newer reports (Guignard & Coles, 1965, Jauhainen et al., 1969), they indicated that whole body vibration had an enhancing effect on auditory function. The effects of vibration on the auditory organ may vary with the experimental condition, it may be impaired, unaffected or improved. Many more studies are necessary before these effects can be elucidated in details.

## RÉSUMÉ

Les changements de sensibilité auditive ont été enregistrés au moyen de la technique de l'audiométrie de Bekesy en exposant à :

- 1° les vibrations de 300 cpm (5 Hz) et 1 000 cpm (16.7 Hz)
- 2° le bruit blanc de 82 dB SL
- 3° les stimulations d'ensemble de 1 et 2°

La vibration de fréquence 300 cpm à 6 mm d'amplitude, ou la vibration de fréquence 1 000 cpm à 3 mm d'amplitude ont été appliquées, jusqu'à ce que le sujet n'ait plus pu les supporter (pendant 20 min) par un excitateur à bruit et vibration contrôlés. On n'a pas trouvé de changements importants à la sensibilité du seuil après avoir exposé qu'aux vibrations. En exposant aux vibrations et au bruit simultanément, les changements du seuil étaient plus grands et le temps de récupération était plus long qu'en exposant seulement au bruit. C'était particulièrement remarquable pour la vibration de 1 000 cpm. On suppose que les effets combinés du bruit et des vibrations pourraient être dus à une ten-

sion physiologique ou à la provision de sang. De nombreuses autres études seront nécessaires pour en déterminer les effets.

## ZUSAMMENFASSUNG

Veränderungen der Hörfähigkeit wurden bei Schwingungen cpm bzw 1000 cpm oder Breitbandgeräusch bei 82 dB SL oder beidem mit einem Bekésy-Audiometer aufgezeichnet. Mit einem Schwingungserreger mit Geräuscheinstellung wurde die Versuchsperson einer Schwingung, Frequenz 300 cpm, Schwingungsbreite 6 mm bzw 1000 cpm zu 3 mm ausgesetzt, solange sie es ertragen konnte, und zwar 20 Minuten.

Bei Schwingungen allein waren keine signifikanten Veränderungen der Hörgrenze zu verzeichnen. Bei Schwingung und zugleich Geräusch entstanden grössere Veränderungen der Hörgrenze (TTS), und die Normalisierung dauerte länger als bei Geräusch allein. Das traf besonders bei einer Schwingung von 1000 cpm zu.

Es ist zu vermuten, dass die Ergebnisse bei Gleich-

zeitung von Schwingung und Geräusch auf eine Störung der Blut-zirkulation sind.

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## SECRETORY PROPERTIES OF CHRONICALLY INFLAMED MIDDLE EAR MUCOSA

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Based on systematic examination of 13 chronically infected temporal bones serially sectioned and stained for mucus the following conclusions concerning the secretory properties were drawn (1) Chronically inflamed middle ear mucosa showed increased secretory activity in the form of goblet and other secretory cells. Large numbers of secreting cells were seen especially in areas of taller epithelium in places mucus positivity could be demonstrated even in the ciliated cells. In certain cases the respiratory epithelium could even be replaced by columnar epithelium. Increased secretory function was observed to be due partly to secretory metaplasia partly to hyperplasia. (2) Glands or gland-like structures were always present but in half the cases only their rudimentary forms were left. The number and activity of glands depended upon the stage of the chronic inflammation. (3) When the situation had become chronic with fibrotic mucosa the secretory elements increased. The mucosa had become fibrotic (healed) even the secretory elements could degenerate and vanish. In cases of chronic non-healed chronic otitis the total number of secretory elements in one ear seemed to amount to thousands, a hundred being active. (4) Clinically grossly diseased middle ear mucosa should be removed during chronic otitis in order to avoid the development of secretory otitis and finally adhesive middle ears.

Despite the assiduous research, opinions on the characteristics of normal middle ear mucosa are partly controversial. Sade (1965, 1966a) and Lim & Huss (1969) using electron microscopy presented evidence that the middle ear lining is a true mucosa and has all secretory elements. On the other hand, Warshawsky (1958) and Tos (1970) claimed that there are no glands in the middle ear mucosa. Infection of the tympanic cavity results in a marked increase of secretory activity of the

mucosa (Friedmann 1956, T. Palva et al., 1964, Lim & Birck, 1971). In Sade's opinion the process was simple secretory hyperplasia while Hentzer (1972) favoured the idea of secretory metaplasia of tympanic epithelium. Great numbers of secretory elements were seen in secretory as well as in chronic (suppurative) otitis media (Sade & Weinberg 1967, Hentzer, 1972). Bak Pedersen & Tos (1973) in their semi-quantitative study, found the greatest density of glands in cases of secretory otitis media while in cases of chronic otitis the density was about 1/3-1/5 of that recorded in secretory otitis.

The purpose of the present work was to study systematically in serial sections the secretory elements of middle ear mucosa in chronically infected temporal bones. Using routine mucus stainings we tried to get a qualitative and semi-quantitative picture of secretory elements and of their relation to tympanic pathology in general in cases of chronic otitis media.

## MATERIAL AND TECHNIQUES

The material consisted of 13 chronically infected temporal bones with ear infections of from 4 months to 70 years duration. The cadavers were kept in cold storage for 13-91 (mean 58 h) hours before the autopsy. The temporal bones were fixed in 10% formalin and decalcified with 22.5% formic acid/sodium citrate solution. After celloidin paraffin double embedding the blocks were sectioned in a frontovertical plane.

This work has been financially supported by Emil Aaltonen Foundation and by the Scientific Fund of the University of Oulu.



Fig. 1 Temporal Anterior lacrimal mucosa at 40 $\times$  magnification. The stratified epithelium contains many goblet cells. The underlying stroma is densely cellular and contains many small blood vessels. One gland is also visible.

and the sectioning was started at the tubal orifice and continued posteriorly to the vertical part of the facial canal. Each 10  $\mu$  thick section stained for mucus, and with Haematoxylin-Eosin (H-E), represented sites 10 mm apart.

In addition to H-E staining for general characteristics, Periodic Acid Schiff (PAS) mucus staining was used, according to the MacManus method. We used this method both with and without diastase digestion. Diastase digestion time varied from 4 to 24 hours. The positive material stained magenta with PAS. Another mucus stain used was Alcian Blue (AB) without hyaluronidase phase. The positive material stained blue with AB.

A more detailed description of the methods and techniques is given in a previous paper (Karma, 1973), based on the same material.

In each section the characteristics of the mucosa and epithelium were examined with a light microscope using magnifications from  $\times 25$  to  $\times 400$ . Special attention was paid to AB-reactivity of epithelium, to the presence of secretory and goblet cells, to the number and amount of glands, cysts and papillae, and to the activity of glandular cells. The thickness, oedema, and vascularity of the mucosa and the general characteristics of the chronic process were noted.

The numbers of glands, cysts and papillae



*Fig 2* Temporal Bone 9 Infero-medial epithelial lining of tubotympanic area. Two goblet cells are seen with a secretory columnar cell between them. The type of the epithelium is pseudostratified ciliated and columnar H.E., 800 oil immersion

semi-quantified by counting the numbers of these structures per section in each individual temporal bone

## RESULTS

### *General characteristics (H-E)*

In previous work mentioned above, Karma (1971) described the general epithelial characteristics of these ears. In cases of chronic infection the middle ear epithelium showed in addition to an increase in its secretory capacity epithelial hypertrophy (Figs 3-4) hyperplasia

(Fig 1) and squamous metaplasia. Immigrating squamous epithelium (Fig 16) epidermization (Fig 16) and even epidermosis were encountered.

As a rule respiratory, often secreting epithelium lined the middle ear cavity widely in its anterior and inferior parts extending in some ears up to the posterior limits of the middle ear cleft. The mucosa showed in all ears thickening, oedema and vascularization of varying degree depending upon the inflammatory state of each ear. Thickness of the mucosa varied from 0.05 to 2.0 mm but was usually about 0.5-1.0 mm.

In addition to mucosal hyperplasia, there was in some cases marked organization, fibrosis and even tympanosclerosis.

In 12 temporal bones the tympanic mucosa was infiltrated by inflammatory cells to a varying extent. The predominant cells were lymphocytes and plasma cells but polymorphonuclear cells were also seen in many ears. Varying numbers of macrophages were observed. Temporal Bone 13 showed the most fibrotic mucosa in the whole material and the active inflammatory process had subsided. This was the only completely dry ear in the material.

#### *Secretory properties (PAS, AB)*

The PAS staining with or without diastase digestion was similar in all sections and the length of the digestion time did not influence the staining. Therefore, when speaking of PAS in the following, this refers to both staining methods.

(a) *Glands* Real glands (Figs 1, 7-10, 19) were seen in 7 ears, the greatest number per section varying from 2 to 30 in each individual ear. The number of glands was greatest antero-inferiorly and medially (especially in the window niches) diminishing postero-superiorly but they could be seen in all tympanic areas. The size of the glands varied, being usually of the order of some tenths of a millimetre and they were usually of simple tubular structure. The proportion of active glands was at least 50% in five cases, in two it was clearly under 50%. In three further temporal bones (1, 9, 11) only some degenerating glandular structures with dilated ducts and stagnant mucus were seen.

(b) *Intra-epithelial glands and epithelial pouches* A few real intra-epithelially located glandular initial stages (Fig. 1) were seen in Temporal Bones 4, 11 and 12, gland-like pouches (Fig. 11) lined by secretory epithelium appeared in 8 other ears.

(c) *Cysts* (Figs 1, 8, 12-15, 19) occurred in all 13 ears and pseudocysts in 11, the maximum of each being up to 50 per section. The infiltration of mucosa by cysts was most pronounced in Temporal Bones 4-11, while pseudo-

cysts were more evenly divided among the ears (see Table 1).

(d) *Goblet cells* (Figs. 2, 3, 11) regularly in 6 ears (Temporal Bones 5, 6, 11, 12, 13) and irregularly in four additional ears.

(e) *Mucoid epithelium* (Figs. 5-8, 11) countered in 4 ears with greater frequency in Temporal Bones 6 and 7. In all ears this change was suggestive but not certain.

(f) *Secretory cells* (Figs. 2-4, 11) Secretory cells in the tympanum were seen in all ears except one. Both columnar and cuboidal secretory cells appeared. Ciliated cells also showed mucus positivity (Fig. 4). AB-positivity was most intense in the ciliated cells (Figs. 3, 4) of the epithelial cells.

(g) *Squamous epithelium* did not show secretory properties (Figs. 14-17). In all ears of squamous epithelial metaplasia. The AB-positive response was demonstrated superficially. However, AB-positivity was limited to the area of slighter metaplasia.

Otherwise, the colour intensity of the staining methods was usually equal. In Temporal Bone 1 PAS positivity was distinctly stronger.

## DISCUSSION

The constant lack of diastase effect in the stainings means that there are no polysaccharides in the mucosa of these sections. It is probable that polysaccharides (including glycoproteins) dissolved from the blocks in the diastase solution.

Fig. 3 Temporal Bone 3. Hyperplastic epithelium from hypotympanum. Goblet cells and secretory cells can be seen. Also some AB-positive material. AB, 600 $\times$  magnification.

Fig. 4 Temporal Bone 3. From the same place as Fig. 3, showing clearly the apically located PAS-positive (secretory) material in the ciliated cells of the respiratory epithelium. PAS, 600 $\times$  magnification.

Fig. 5 Temporal Bone 6. Mucous epithelium on the antero-medial wall. AB, 240 $\times$  magnification.

Fig. 6 Temporal Bone 6. From the same place as Fig. 5, showing mucous epithelium. PAS, 240 $\times$  magnification.

Fig. 7 Temporal Bone 6. Active glandular structures in the wall of tympanum. PAS-positive material in a small duct to tympanic lamina. Temporal Bone 6. Mucosally charged. PAS, 96 $\times$  magnification.

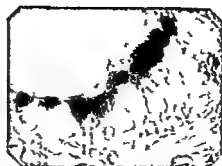
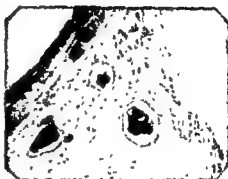
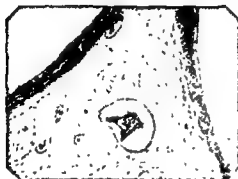


Fig 8 Temporal Bone 6 Infero-medial wall is lined by mucous epithelium. Below a simple tubular gland is and above it two cysts. The mucosal stroma has no positivity PAS  $\times 96$   
 Fig 9 Temporal Bone 4 A simple gland from infero-lateral wall of posterior tympanum. The stroma is relatively fibrotic and devoid of PAS positive material  
 150

Fig 10 Temporal Bone 7 A gland from medial tympanic wall in AB-staining AB  $\times 150$   
 Fig 11 Temporal Bone 6 Infero-medial wall of anterior tympanum. Mucous epithelium with gland like pouch formation. Subepithelial stroma is relatively fibrotic HE,  $\times 400$





*Fig. 12. Temporal Bone 5. Cystic formations in the attic. AB, x24.*

*Fig. 13. Temporal Bone 5. The same area as in Fig. 12, in PAS-staining. x24.*

*Fig. 14. Temporal Bone 12. Down from the inferior perforation edge. On the ear canal side, the epidermis does not stain with AB. Cysts, tympanal epithelium and secretory blanket above it stain blue (AB-positive). AB, x96.*

*Fig. 15. Temporal Bone 12. The same area as in Fig. 14. The PAS-staining is identical with AB. PAS, x96.*

*Fig. 16. Temporal Bone 8. In the upper part of the tympanum (near oval window area) the epidermis changes to low columnar type. The epithelial junction is abrupt. The epidermis is AB-negative but ordinary tympanal dermis is AB-negative but ordinary tympanal dermis stains abundantly with AB. Medial to there is a cyst having a faint bluish shade. AB, x40.*

*Fig. 17. Temporal Bone 12. The lower part of the ear canal. The PAS-negative epidermis of the ear canal is abruptly at the border to temporal PAS-positive epithelium. PAS, x240.*

tages. Consequently, the PAS positive reaction is obtained only from the carbohydrate-complexes.

generally equal coloration with AB and stains suggests that the secretion has a mucous quality (Shackleford & Klapper, 1963). In those areas where PAS positivity was greater than AB serous secretion with mucous substances apparently predominated all degenerating and active glands as well as intra-epithelial glandular structures were found. The total number of gland like formations was at the most to 110 per section in Temporal Bone 8 (see Table I). This means that the number of glandular processes in the entire middle ear area amounts to several thousands. On the other hand, in Temporal Bone 13 there were only a few visible cysts as a sign of former secretory activity. In cases of secretory otitis

Bak Pedersen (1973) counted the average number of glands (all degenerative forms included) in the whole tympanic area and arrived at a figure of about 2000, 93% of the glands being active. In their cases of chronic otitis the mean density of the glands was 18% of the former and the percentage of active glands was only 8-20 of the total (Bak Pedersen, 1973). In the present material the number of glandular structures and the proportion of active glands varied so greatly (see Table I) that no meaningful average numbers can be given. The reason for this great variation is the fact that our material included ears in very different stages of chronic processes, from dry ears to purulent oedematous mucosa. However, the general impression was that in non-degenerating ears the total number of all glandular structures was some thousands and active glands some hundreds per ear.

The greatest number of glands was usually found in areas with marked mucosal hyperplasia

(Figs 1, 19). Goblet and other secretory cells also appeared in larger numbers in the areas showing hyperplastic mucosa. On the other hand, the ears with most active inflammation (Temporal Bones 1-3) and also showing pronounced mucosal oedema, had remarkably few secretory elements. Ears with fibrotic mucosa (Temporal Bone 13) and ears with large areas of squamous epithelial lining (cf Karma, 1973) had only few secretory structures. Thus in the acute stage (here acutization of a chronic process) the discharge is a result of both transudation and exudation. The increase of secretory elements takes place predominantly when the inflammation has calmed down, resulting in both hyperplastic subepithelial stroma and epithelium.

Cysts and pseudocysts were more numerous than the glands and they were more evenly distributed throughout the whole material. This is explained by the long standing ear disease. Over the years, the active glands had degenerated, after acute exacerbations new glands had formed and again degenerated thus increasing the number of remarkably stable cystic structures. In granulating areas many pseudocysts had formed while the flat tympanic epithelium attempted to cover the raw surfaces but lost the fight and was buried in the stroma by overgrowing granulation tissue. After cessation of inflammation, cysts and pseudocysts may also lose their lumen and even the remaining epithelial strings may degenerate and vanish (see Temporal Bone 13).

Goblet and other secretory cells (see Table I) were usually seen in the areas with taller epithelium and in places the respiratory epithelium was wholly replaced by mucoid epithelium. This means that there developed a change from the original function of transportation to secretion in the function of the epithelium. Also ciliated cells showed mucous positivity in some areas especially in the apical parts of the cells (Fig. 4). Both these findings suggest a mechanism of secretory metaplasia in cases of chronic otitis, in other words a metaplastic change of ciliated (and other epithelial) cells to secreting

\* Temporal Bone 7 Tympanic respiratory epithelium shows numerous goblet and columnar secretory cells.  $\times 240$ .

† Temporal Bone 8 Oval window area. There are many large and more peripherally cystic structures in hyperplastic mucosa. PAS,  $\times 40$ .

Table 1 A synopsis of the findings

Only positive findings are noted

The temporal bones have been numbered beginning from the ear showing most active inflammation and ending in the only dry ear (in which the active process is over) of the material. The identification of respective ears in the earlier work (Karma, 1973) is given within parentheses. The proportion of active glands (a very rough proportion) is in the so-called 'transitional varieties' (Tos & Bak-Pedersen, 1972), cysts and pseudocysts (Döderlein, 1920) are not counted to the proportion

Temporal Bone	1(1)	2(2)	3(1)	4(5)	5(4)	6(7)	7(8)	8(12)	9(13)	10(11)	11(10)	12(6)	13(9)
Age	70	56	85	59	59	70	51	83	58	83	51	11	70
Duration of symptoms					Minimum		From	childhood		1 yr	childhood		
Total absence of drum					4 months								
Total defect of tensa													
Subtotal defect of tensa													
Central perforation													
Dry tympanum													
Moist tympanum													
Purulent tympanum													
Thickness of mucosa, mm													
Mucosal oedema													
Mucosal vascularization													
Secretion (AB & PAS +) in tympanum													
Inflammatory cell infiltration (degree of positivity is not evaluated)													
Follicular lymphocyte accumulations (degree of positivity is not evaluated)													
Cilia (number of strips)													
Epithelial AB & PAS positivity													
Secretory cells													
Golgi cells													
Glands (ad number section)													
Proportion of active glands													
Cysts (ad number section)													
Pseudocysts (ad number section)													
Intraepithelial glands (p - as pseudocyst sections, B - ad glands sections)													
Ad - not epithelioid													
Ad - not epithelioid													
Ad - not epithelioid													

which mechanism was already proposed soendlin (1959) and later confirmed by electron microscopic findings of Lim & (1971) and Hentzer (1972). However, during the very great number of secretory and glandular structures in some of our ears compared with the smaller number of cells in normals (Sade, 1966a), secretory granulation must occur in addition to secretory metaplasia in cases of chronic otitis.

These data on serial sections from chronically inflamed middle ear mucosa have important clinical implications. On the basis of the removal of squamous keratinizing epithelium, Palva et al., 1968, Karma, 1973) have emphasized the need for its complete removal from middle ear. We have felt, however, that in the case of non-keratinizing squamous epithelium, difficult to recognize without microscopic examination, such thorough removal is required since the possibly remaining small islands of squamous metaplasia may revert to normal middle ear mucosa so that no cholesteatoma develops.

The present findings of thick mucosa, covered mucosally changed epithelium and containing in some ears thousands of glands, cysts and pseudocysts in the stroma, suggest that this mucosa cannot return to normal over time and probably never does. In other words, the presence of this type of tympanum with a new drum, even supposing that thorough removal of the mastoid and epitympanic space has been made, is likely to lead to accumulation of secretions in the middle ear space analogous to the cases of secretory otitis media. During the repair, part of the middle ear mucosa is also traumatized with resultant raw areas. In the presence of fibrin in the thick seromucinous fluid, the glands inevitably leads to the development of adhesive middle ears, or to non-healing of the graft.

Having witnessed the frequent development of adhesive middle ears we have for some years recommended removal of grossly diseased thickened mucosa from the promontory, and have covered the raw areas with Silastic sheet-

ing. Although we have not had a chance to study any of these ears later in serial sections, it may be presumed that a flat 1-2 cell layer of epithelium grows to cover the denuded areas under the Silastic. Transportation of some secreted material, possibly formed in the hypotympanum or the tympanic roof, may occur by means of the cilia strips that may grow in the areas not covered with Silastic sheeting. Similarly, thorough removal of suspected secretory epithelium from the posterior tympanum is mandatory as these secretions would not find their way out to the Eustachian tube in reconstructed and Silastic sheeting-covered middle ears.

It has been suggested that the secretory activity of the middle ear mucosa after drum reconstruction may cease after insertion of grommets like those in the treatment of secretory otitis media. In our experience this is not true and use of middle ear ventilation tubes is successful in the long run only if the major part of the pars tensa with its elastic layer is intact. Also, mucosal changes in secretory otitis media are both by gross examination and in microscopic study considerably less pronounced than those of chronic long standing otitis media, and the time needed for the return to normal of the former may be several years. Removal of the diseased mucosa is then on the way to healing.

## ZUSAMMENFASSUNG

An 13 chronisch entzündeten Schläfenbeinen wurden Serienschritte gemacht und eine Schleimfärbung durchgeführt. Aus ihrer systematischen Untersuchung konnten die folgenden Schlüsse über die sekretorischen Eigenschaften der Schleimhaut gezogen werden:

1 Chronisch entzündete Mittelohrschleimhaut zeigte ein erhöhtes Sekretionsvermögen in Form von Becherzellen und anderen sekretorischen Zellen. Eine grosse Zahl sezernierender Zellen wurden speziell in den Bereichen des höheren Epithels festgestellt. Stellenweise konnte ein positiver Schleimnachweis auch an Zilien tragenden Zellen gezeigt werden. In bestimmten Bereichen konnte das respiratorische Epithel auch durch mukoses Epithel ersetzt sein. Vermutlich beruhte die erhöhte sekretorische Funktion teils auf sekretorischer Metaplasie und teils auf sekretorischer Proliferation.

2 Drüsen oder drüsenartige Strukturen waren immer vorhanden, aber in der Hälfte der Fälle fanden sie sich

nur in degenerierten Formen Zahl und Aktivität der Drüsen hingen von dem Stadium des chronischen Prozesses ab. Wenn die Situation chronisch geworden war, nahmen mit der hyperplastischen Schleimhaut die sekretorischen Elemente zu. Wenn die Schleimhaut fibrotisch geworden war (abgeheilt war), kam es auch zu Degeneration und Schwund der zystischen Elemente. In Fällen hyperplastischer, nichtverheilter chronischer Otitis schien die totale Zahl der Drüsen in einem Ohr auf Tausende angewachsen zu sein, von denen ein paar Hundert aktiv waren.

3 Klinisch gesehen sollte Promotoriumschleimhaut mit ausgeprägten Krankheitszeichen bei der Chirurgie der chronischen Mittelohrentzündung entfernt werden, um die Entwicklung einer sekretorischen Otitis und im weiteren von adhäsiven Mittelohrprozessen zu vermeiden.

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## FURTHER STUDIES ON ALTERNOBARIC VERTIGO

*Posture and Passive Equilibration of Middle Ear Pressure*

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(Received February 12 1974)

The passive equilibration of the middle ear during simulated ascents was affected by different  $P_{tm}$ s and the level of the relative overpressure in the ear ( $P_{tm}$ ) required to force the Eustachian tubes was higher in the recumbent than in the sitting position. Subjects with a high forcing pressure (FPL) in the ear and with experience of Alternobaric Vertigo exposed to simulated ascents in different postures had a  $P_{tm}$  recorded at the moment of onset of nystagmus (nystagmus pressure level NPL) was also higher in the recumbent position. However an initially high FPL increased as regularly as an initially low FPL in the absence of vestibular stimulation in some subjects examined in the recumbent position was explained by the fact that the FPL did not reach the NPL. A relation between the middle and the inner ear pressure being the basis for vestibular stimulation is discussed.

It is well known that the pressure equalizing function of the Eustachian tubes is affected by different positions of the body or the head (Hartman 1866) and Hartman (1878) studied this phenomenon by recording the pressure level required to open the tubes during Valsalva's manoeuvre. Perlman (1939) studied the influence of different body positions on the patency of the Eustachian tubes of patients with tuba aperta, and found a reduced patency in the recumbent position as compared with the erect position. The reduced patency was assumed to be caused by lymphatic venous stasis in the vessels of the tubal mucosa. The reduction of the pressure regulating function of the tubes in the recumbent position

has later been confirmed by Rundcrantz (1969) in an investigation of subjects with eardrum perforation due to chronic otitis media or to trauma. Ingelstedt et al (1967) could demonstrate the same phenomenon even in healthy military aircraft pilots.

The assumption that venous stasis explains the reduced patency in the recumbent position was supported by a study of the pressure in the bulbous vena jugularis in different postures (Jonsson & Rundcrantz, 1969). The pressure measured on the same level as the Eustachian tube was found to be about zero with the subjects seated and about 10 cm H<sub>2</sub>O with the subjects in the recumbent position. Ingelstedt et al (1967) also found that a pressure increase inside the internal jugular vein caused an increase of the volume of the middle ear mucosa as an expression of vascular filling of the mucosa. A corresponding volume increase of the tubal mucosa might consequently be a possible explanation of the reduced patency of the Eustachian tubes in the recumbent position or during neck vein compression.

It has been demonstrated that vertigo might be elicited in otologically healthy subjects by a relative overpressure of a certain level, induced in the middle ear by lowering of the ambient pressure (Ingelstedt et al, 1974). The subjects were instructed to avoid active clearing of the middle ear pressure during the ambient pressure decrease, and the magnitude of the induced overpressure was dependent upon the level of

This investigation was supported by grants from the

the passive forcing pressure, i.e. the pressure required to force the Eustachian tubes open passively. In the same work asymmetry was also demonstrated between the forcing pressure level on the right and the left hand side. The importance of the magnitude of this asymmetry for the vestibular stimulation could, however, not be evaluated.

The aim of the first part of the present work was to study the influence of different body positions on the passive equilibration of the middle ear pressure at simulated ascents.

## METHOD AND EQUIPMENT

The following symbols are used in text and figures

$P_{atm}$	atmospheric pressure on ground
$P_m$	pressure in middle ear
$P_{tm}$	pressure gradient across eardrum
$P_{ch}$	pressure in chamber, i.e. ambient pressure
$V_m$	volume of airfilled middle ear space
$I_{tm}$	volume displacement of eardrum in relation to its neutral position
$V_{tm}$	airflow through resistor of the ear canal flowmeter caused by volume displacement of eardrum
$I_{ac}$	airflow through resistor of the ear canal flowmeter, caused by expansion and compression of gas volume in external ear canal and in flowmeter system by changing the ambient pressure
$I_{ref}$	airflow through resistor of the reference flowmeter, caused by expansion and compression of gas volume in reference system by changing the ambient pressure

$\Delta$  before the symbol indicates a change of the variable. Pressure is expressed in cmH<sub>2</sub>O, volume in microlitres ( $\mu$ l) or millilitres (ml) and airflow in microlitre/sec ( $\mu$ l/sec).

$P_m$  and  $P_{ch}$  are relative to atmospheric pressure on the ground.

Fig. 1 gives an outline of the equipment used for the recordings. A pressure chamber was used in which the subjects were exposed to simulated ascents of 90 cmH<sub>2</sub>O during which they were instructed to avoid active equilibration of the middle ear pressure. The ambient pressure decrease caused a relative overpressure in the middle ear and there was no pressure equilibration until the relative overpressure was great enough to force the Eustachian tubes open passively (forc-

ing pressure level FPL). A method (Ingelstedt et al., 1967; Elner et al., 1971) in which it is possible to record the volume movement of the eardrum ( $I_{tm}$ ) caused by the overpressure in the middle ear (Fig. 1) made it possible to determine the pressure change in the chamber ( $\Delta P_{ch}$ ) to force the tubes open passively. A polycatheter with a rubber disc was inserted into the inner bony part of the external ear canal tightly connecting the space between the eardrum and the disc with the measuring device. This method is based on recording the airflow through the eardrum displacement ( $I_{tm}$ ) by means of a flowmeter. The airflow through the flowmeter consists both of the flow  $I_{tm}$  and  $V_{ac}$  has to be eliminated for the recording of  $V_{tm}$ . This elimination is possible by using an identical flowmeter with an identical reference volume  $V_{ref}$  is then integrated and recorded.

The relative overpressure in the middle ear at the moment just before the tube is forced open is, however, not equal to the pressure change in the chamber ( $\Delta P_{ch}$ ) at the same moment. It has been shown that there is only a small difference between  $P_m$  and  $\Delta P_{ch}$  in the case of clinically healthy subjects, i.e. subjects without middle ear disease or a large middle ear volume (>6 ml) (Elner et al., 1974).

In the first part of the present study the middle ear pressure was given as  $\Delta P_{ch}$ . For a determination of the pressure gradient across the eardrum ( $P_{tm}$ ) at the moment of passive forcing it is necessary to know  $\Delta P_m$  as well as  $\Delta P_{ch}$  induced during the simulated ascent and at the moment of passive forcing. This can be calculated as  $\Delta P_{ch} - \Delta P_m$ . For a description of the method and the calculation of  $\Delta P_m$ , see Ingelstedt et al. (1967) and Elner et al. (1971a).

Eye movements were recorded simultaneously with the pressure recordings by means of nystagmography (ENG) and the recordings were performed in total darkness with the subjects' eyes open (Tjernström 1973). The subjects were also instructed to report any feelings of

**I Forcing pressure level (FPL) of the right and the left ear, recorded during simulated ascents**  
 passive clearing of the ears in the sitting, the recumbent and the left and the right lateral supine positions

<sup>1</sup> PL is given as  $\Delta P_{ch}$ , i.e. the pressure change in the chamber required to induce a relative overpressure in the ears high enough to force the tubes open passively

No.	Position	Right ear			Left ear		
		FPL ( $\Delta P_{ch}$ , cmH <sub>2</sub> O)		No of exams	FPL ( $\Delta P_{ch}$ , cmH <sub>2</sub> O)		No of exams
		Mean	Range		Mean	Range	
1	Seated	31.3	28.5-34.5	9	18.4	11.0-19.5	9
	Recumbent	46.0	43.5-48.0	10	35.0	33.0-37.5	10
	Right lateral	50.3	48.0-52.5	2	36.0	35.5-36.5	2
	Left lateral	48.0	45.0-51.0	3	40.5	36.0-43.5	3
2	Seated	37.7	32.0-43.0	11	49.0	41.0-58.0	11
	Recumbent	51.5	49.0-53.0	5	60.2	57.0-65.0	5
	Right lateral	57.0	56.0-58.0	3	60.0	58.0-62.0	3
	Left lateral	51.3	49.0-53.0	3	64.7	63.0-66.3	3
3	Seated	35.7	34.0-38.0	11	31.3	26.0-35.0	11
	Recumbent	57.5	53.0-60.0	6	51.3	46.0-54.0	6
	Right lateral	65.5	64.5-66.5	2	48.5	46.0-51.0	2
	Left lateral	58.5	58.0-59.0	2	62.5	61.0-64.0	2
4	Seated	41.3	38.0-45.0	9	33.2	31.2-36.0	9
	Recumbent	53.0	52.0-54.0	4	44.0	42.0-46.0	4
5	Seated	42.8	39.0-46.0	11	38.9	34.0-46.0	11
	Recumbent	51.6	50.0-53.0	5	45.2	43.0-46.0	5

The flow-volume measuring unit and the pressure measuring unit were calibrated before and after every examination according to the standard method. Eye-movements were calibrated for 30° in each direction before and after the test.

#### Experimental procedure

The subjects were exposed to simulated ascents and passive clearing of the ears. The examinations were performed with 5 subjects in the sitting and the recumbent position. Furthermore, the subjects were also examined in the left and right lateral supine positions. The subjects were held in each position for about 5-10 minutes before the examination started.

#### MATERIAL

The material consisted of 5 healthy subjects in the age range 22-34 years, 2 of whom women and 3 men. All subjects were regarded as otologically

normal with no history of ear disease and a normal ear examination. The hearing threshold was within 0-20 dB (related to ISO standard, 1964). All subjects were without signs of catarrhal infection at nose and throat examination. The subjects had a normal active tubal function (groups Ib and Ic, according to Eimer et al., 1971b). All of them were also known to have a forcing pressure of a normal level (30-50 cmH<sub>2</sub>O, see Ingelstedt et al., 1974). They all had a middle ear volume of both ears >6 ml.

#### RESULTS

In Table I the FPL of right and left ears are given, recorded with the subjects in different postures. The FPL was found to be higher in the recumbent than in the sitting position. The mean increase was about 13 cmH<sub>2</sub>O (mean of all means). The FPL was also affected by the different lateral positions and the recordings showed a higher FPL in the ear which was turned down-



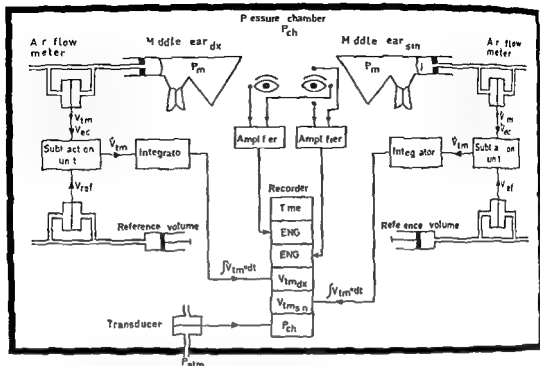


Fig 1 Outline of the equipment used for recordings of eardrum displacement ( $V_{tm}$ ) at changing ambient pressure ( $P_{ch}$ ). The equipment as well as the subjects are placed in

the pressure chamber. For more details see text and symbols.

wards than in the ear turned upwards. No sign of vestibular stimulation was observed.

### COMMENT

The results presented in Table I which show that a higher FPL is required to force the tubes open in the recumbent position, are in agreement with earlier investigations into the patency of tubes in different positions (Lucae, 1866, Hartman, 1878, Perlman 1939, Ingelstedt et al, 1967, Runderantz 1969). However, there seems to be no published report of the magnitude of the FPL increase due to changes of posture. It is also seen from the results that it might be possible to vary the FPL asymmetry between the ear by examinations in different lateral positions.

It has been demonstrated (Ingelstedt et al, 1974) that certain subjects have a particularly high FPL in one ear and a lower FPL in the other. These pressure conditions were proved not to be chance findings as it was possible to

reproduce them at repeated examinations at intervals of several months. 5 subjects with a high FPL ( $> 60 \text{ cmH}_2\text{O}$ ) reported vertigo when exposed to simulated ascents and descents. Clearing of the ears in the sitting position. These 5 subjects reported vertigo during ascent and 3 only occasionally. Nystagmus appeared every time vertigo was reported. Nystagmus was also observed in several subjects although the subjects did not recognize vestibular stimulation. One subject never reported vertigo and no nystagmus was observed although this subject also had a high FPL.

The aim of the second part of the work was to study

(a) the possibility of increasing eardrum forcing pressure by change of body posture to find out if a higher relative overpressure in the middle ear causes a stronger vestibular response,

(b) if a subject with a high forcing pressure level but without feelings of vertigo in the sitting position gets vertigo and/or nystagmus in the

## II Forcing pressure level (FPL) of the right and the left ear, recorded during simulated ts and passive clearing of the ears in the sitting and the recumbent positions

Position	Right ear			Left ear			Vertigo incidents/ No of ex	Nystagmus incidents/ No of ex	Velocity of slow nystagmus phase (°/sec)
	FPL ( $P_{tm}$ , cmH <sub>2</sub> O)		No of exams	FPL ( $P_{tm}$ , cmH <sub>2</sub> O)		No of exams			
	Mean	Range		Mean	Range				
Seated	58.4	54.6-62.4	3	37.6	33.9-40.7	3	3/3	3/3	0.5-1.0
Recumbent		67.9	1		59.6	1	1/1	1/1	2.0
Recumbent + neck compression	73.7	71.8-75.3	3	—	—	—	3/3	3/3	1.0-2.0
Seated	63.0	61.3-65.6	5	50.9	46.0-56.0	5	0.5	0.5	—
Recumbent	72.4	69.9-74.5	7	62.0	60.3-66.1	6	1/7	5/7	1.5-3.0
Seated	70.6	65.4-74.1	6	47.6	43.1-50.2	6	1/6	1/6	0.5
Recumbent	72.6	67.3-79.3	4	56.2	53.0-58.7	4	0.4	0.4	—
Seated	70.4	68.1-73.4	9	47.6	38.6-54.4	9	1/9	3/9	0.5-1.5
Recumbent	74.5	71.8-76.5	3	54.4	46.9-66.5	3	0.3	0.3	—
Seated	63.7	62.5-65.4	5	34.2	33.2-46.7	7	3/7	3/7	1.0-1.5
Recumbent	63.1	61.1-66.9	3	52.0	49.6-55.3	3	0.3	0/3	—
Seated	60.8	59.4-62.2	3	66.1	65.2-67.9	3	1/3	2/3*	2.0-3.5
Recumbent	58.6	57.8-59.4	2	60.1	55.2-65.0	2	0.2	0/2	—

recumbent position when the forcing pressure is higher,

(2) the possibility of inducing a variation in the forcing pressure asymmetry between the left and right ear in order to evaluate the importance of the asymmetry in the mechanism of Alternobaric Vertigo

### Method, equipment and experimental procedure

The method is described above and the equipment is presented in Fig. 1. The FPL was given by  $P_{tm}$  and the calculations, which require knowledge of the middle ear volumes ( $V_m$ ), were performed as described by Ingelstedt et al (1974). The subjects had a middle ear volume  $>6$  ml, determined indirectly by converting the area of an air-filled middle ear system, measured from X-ray films, to a volume (Andreasson, 1973). The subjects were exposed to simulated ascents in the sitting and the recumbent positions. One subject was also examined in the left- and the right-hand lateral position, and in two experiments (see Results) the neck veins were com-

pressed by means of an inflatable cuff (Jonson & Rundcrantz, 1969). The cuff was inflated to 40 cmH<sub>2</sub>O by a pressure fan.

**Material** The material consisted of 6 healthy subjects aged between 22 and 26 years, all of whom were men. The subjects were known to have a high FPL on one side ( $>60$  cmH<sub>2</sub>O) in the sitting position, and a forcing pressure asymmetry between the ears which differed from one subject to another. All subjects were regarded as otologically normal with no history of ear disease and a normal ear examination. The hearing threshold was within 0-20 dB (related to ISO standard, 1964). The subjects were without signs of catarrhal infection at nose and throat examination and there was no history of labyrinthine disease. The subjects had a normal active tubal function (groups Ib and Ic, according to Elner et al, 1971b). All subjects but one (subject VII) had reported vertigo when exposed to simulated ascents and passive clearing of the ears in the seated position. Subject VII had had

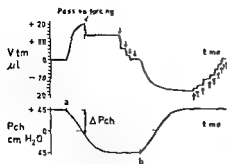


Fig 2 Recording of eardrum displacement ( $V_{tm}$  in  $\mu l$ )

ear pressure is equilibrated with the chamber pressure ( $V_{tm}=0$ ). Then the examination starts (a) with a simulated ascent (pressure decrease of 90 cmH<sub>2</sub>O). The Eustachian tube is forced open passively (passive forcing) the relative overpressure in the middle ear is then reduced (the eardrum moves inwards) and the middle ear pressure is equilibrated to the chamber pressure by deglutitions (arrows). Before the start of another examination the chamber pressure is increased (b) and the eardrum moves inwards due to a relative underpressure in the middle ear. The underpressure is equilibrated with the chamber pressure by deglutitions (arrows) and it is checked that the eardrum is in its neutral position before start of the next examination.

no feeling of vertigo despite a high forcing pressure on one side.

**Results** The relative overpressure in the middle ear ( $P_{tm}$ ) was calculated at two different moments during the ascents, i.e. at the moment when the Eustachian tube was forced open passively (FPL) and at the moment when the onset of nystagmus was recorded (NPL = nystagmus pressure level). In Table II the FPL on the right and the left hand side is given, in the sitting and the recumbent positions, together with the number of vertigo incidents and the intensity of the vestibular response (velocity of the slow nystagmus phase).

In subjects VI and VII a bilaterally higher FPL in the recumbent position was recorded and the increase was of about the same magnitude as in the subjects presented in Table I. The recordings of subjects VIII and IX showed a bilaterally higher mean FPL in the recumbent position. However, some overlapping isolated results were also seen. The rise in FPL was less

pronounced in the ear with a high FPL (left ear) than in the ear with a low FPL (right ear). In subject X the mean FPL was found about the same in the right ear (high) irrespective of posture while an FPL was recorded on the left hand side (low) in the recumbent position. Subject XI had FPL on both sides when examined in the sitting position, but the posture change did not show any increase in FPL. On the contrary the findings showed a lower value of the FPL in the recumbent position.

In all subjects but one (subject VII) no vertigo as well as vertigo appeared when the subjects were examined in the sitting position. Subject VII reported vertigo in both positions (sitting and recumbent) and the vestibular response was even to be a little stronger in the supine position. No sign of vestibular stimulation appeared when subject VII was examined in the sitting position, but in the recumbent position vertigo was reported once and nystagmus appeared

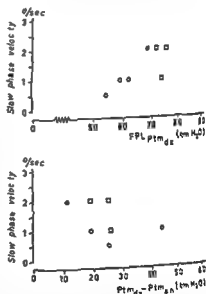


Fig 3 Relation between the intensity of the vestibular response (max velocity of the slow nystagmus phase) and the forcing pressure level (FPL) (upper part). Relation between the intensity of the vestibular response and the pressure asymmetry between the right and the left ear ( $P_{tm_d} - P_{tm_s}$ ) at the moment when the slow nystagmus phase was recorded (lower part). The sitting position is marked with circles and the recumbent position with squares.

examinations Subjects VIII, IX, X and XI tested no vertigo when examined in the recumbent position and no nystagmus appeared. Subject VI was also examined in the recumbent position during neck vein compression in order to induce an additional FPL increase, which was observed, and both nystagmus and vertigo appeared. Subject VII reported vertigo when examined in the left- and right-hand lateral positions and nystagmus was also recorded. The results of the examinations of subjects VI and VII, all performed at one session, are presented in Figs 3 and 4. The upper half of the figures shows the relation between the vestibular response (nystagmus) and the FPL. The lower halves show the relation between the pressure asymmetry ( $P_{\text{left}} - P_{\text{right}}$ ) (difference between the relative overpressure in the right and the left middle ear,  $P_{\text{middle ear}} - P_{\text{atmosphere}}$ , at the moment when the right Eustachian tube is forced open) and the vestibular response.

Although the findings are not statistically conclusive, the graphical presentation in Figs 3 and 4 seems to indicate a stronger vestibular response at higher FPL. It is also indicated that the magnitude of the pressure asymmetry between

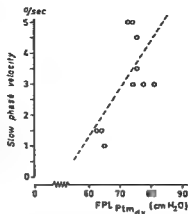
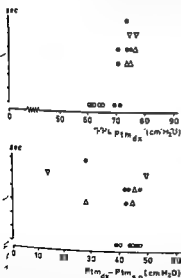


Fig. 5 Relation between the intensity of the vestibular response and the forcing pressure level (FPL). The figure shows the examinations of subject X, performed in the seated position at different sessions. The line of short dashes represents a calculated regression line.

the ears did not seem to affect the intensity of the vestibular stimulation when the asymmetry exceeded about 10 cmH<sub>2</sub>O.

It seemed surprising that no sign of vestibular stimulation was recorded when subjects VIII, IX and X were examined in the recumbent position, in spite of the fact that in this position the recorded FPL was apparently high enough to stimulate the vestibular system in the sitting position. In view of these results, the pressure level required to cause a vestibular stimulation in the recumbent position seemed to be higher than that in the sitting position. In order to test this assumption the NPL recorded in the sitting and the recumbent positions of subject VI was calculated. This calculation showed that the mean NPL was about 8 cm higher in the recumbent position than the mean NPL in the sitting position, and the NPL was even higher when the examinations were performed in the recumbent position during neck vein compression. In order to confirm the results presented above, repeated examinations of subjects VI, VII, VIII and X were performed in the sitting and the recumbent position, and subject VII was also examined during neck vein compression in the recumbent position.

Table III shows the results of the re-examinations and the FPL is given for the ear with the



The figure shows examinations of subject VII all of which were performed at one session, and the same positions are presented as in Fig. 3. O = seated position, ■ = recumbent position, Δ = right lateral supine position, ▽ = left lateral supine position.

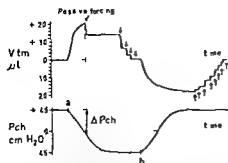


Fig 2 Recording of eardrum displacement ( $V_{tm}$  in  $\mu l$ ) and chamber pressure ( $P_{ch}$  in  $cmH_2O$ ). Eardrum movement outwards (+) and inwards (-). Each examination starts with a simulated descent with a pressure increase of  $+45 cmH_2O$  after which it is checked that the middle ear pressure is equilibrated with the chamber pressure ( $V_{tm} = 0$ ). Then the examination starts (a) with a simulated ascent (pressure decrease of  $90 cmH_2O$ ). The Eustachian tube is forced open passively (passive forcing) the relative overpressure in the middle ear is then reduced (the eardrum moves inwards) and the middle ear pressure is equilibrated to the chamber pressure by deglutitions (arrows). Before the start of another examination the chamber pressure is increased (b) and the eardrum moves inwards due to a relative underpressure in the middle ear. The underpressure is equilibrated with the chamber pressure by deglutitions (arrows) and it is checked that the eardrum is in its neutral position before start of the next examination.

no feeling of vertigo despite a high forcing pressure on one side.

**Results** The relative overpressure in the middle ear ( $P_{tm}$ ) was calculated at two different moments of the ascents, i.e. at the moment when the Eustachian tube was forced open passively (FPL) and at the moment when the onset of nystagmus was recorded (NPL, nystagmus pressure level). In Table II the FPL on the right and the left hand side is given, in the sitting and the recumbent positions together with the number of vertigo incidents and the intensity of the vestibular response (velocity of the slow nystagmus phase).

In subjects VI and VII a bilaterally higher FPL in the recumbent position was recorded and the increase was of about the same magnitude as in the subjects presented in Table I. The recordings of subjects VIII and IX showed a bilaterally higher mean FPL in the recumbent position. However, some overlapping isolated results were also seen. The rise in FPL was less

pronounced in the ear with a high FPL (left ear) than in the ear with a low FPL (right ear). In subject X the mean FPL was lower, about the same in the right ear (left ear), irrespective of posture, while an FPL was recorded on the left hand side (low) in the recumbent position. Subject XI had FPL on both sides when examined in the recumbent position, but the posture change did not cause any increase in FPL. On the contrary the recordings showed a lower value of the FPL in the recumbent position.

In all subjects but one (subject VII) no vertigo as well as vertigo appeared when the subjects were examined in the sitting position. Subject VII reported vertigo in both positions (recumbent) and the vestibular stimulation even to be a little stronger in the supine position. No sign of vestibular stimulation appeared when subject VII was examined in the sitting position but in the recumbent position vertigo was reported once and nystagmus appeared

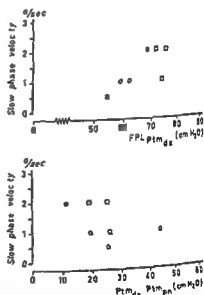


Fig 3 Relation between the intensity of the response (max. velocity of the slow nystagmus phase) and the forcing pressure level (FPL) (upper part). Relation between the intensity of the response and the pressure asymmetry between the right and left ear ( $P_{tmr} - P_{tlml}$ ) at the moment when the Eustachian tube is forced open. The following examinations of subject VI, all of which were at one session:  $\circ$  = sitting position,  $\bullet$  = recumbent position and neck vein occlusion,  $\square$  = recumbent position and neck vein occlusion.

required to cause a vestibular stimulation in the recumbent position, as the tubes forced open passively by a lower pressure than the NPL. However, as is seen from the III the FPL of subject X was much higher in one of the sessions, and in this case nystagmus as well as vertigo appeared in the recumbent position, too. The same was also observed in subject VIII in one of the re-examinations.

Fig. 5 gives a graphical presentation of all examinations of subject X performed at different times in the sitting position. The figure shows a variation between the FPL and the intensity of the vestibular response (velocity of the slow nystagmus phase).

## DISCUSSION

The pressure level required to force the Eustachian tube open passively was affected by different positions of the body in agreement with the pressure effects on the tubal function (Ingelstedt 1967, Rundcrantz, 1969). It seems probable that the postural effects on the FPL is due to a change of vascular filling of the tubal mucosa due to a changed hydrostatic venous pressure in the recumbent position (Jonson & Rundcrantz, 1969).

This assumption is confirmed by the experiments with neck compression, which caused an additional increase in the FPL. It seems reasonable to suppose that the postural effects on the tubal mucosa is similar to that on the middle ear mucosa (Ingelstedt et al., 1967, Rundcrantz, 1969). However, a change of posture did not affect an initially high FPL as regularly as an initially low FPL. In one subject a slight FPL decrease was observed in the recumbent position.

The level of the relative overpressure in the middle ear ( $P_{rel}$ ), recorded at the moment of onset of nystagmus (NPL), was, however, also found to be higher in the recumbent than in the sitting position. An additional NPL increase was furthermore observed when the examinations were performed during compression of the neck

veins. The NPL increase seemed to be even more regular than the FPL increase.

The intracranial pressure is known to increase by about 10–13 cmH<sub>2</sub>O when the posture is changed from the erect to the horizontal position (Best & Taylor, 1945). If the cochlear and/or the endolymphatic ducts have a pressure-equalizing function (Allen, 1964, House, 1964, Holden & Schuknecht, 1968), the intralabyrinthine pressure would be expected to rise when the intracranial pressure is increased. Since the increase of the intracranial pressure is mainly an effect of a hydrostatic venous pressure increase (Dawson, 1967), it might be assumed that the intralabyrinthine pressure would also rise as a result of the same mechanism even without any pressure-equalizing function on the part of the ducts.

In a previous study (Tjernström, 1974) it was found possible to elicit approximately the same vestibular response by overpressure in the middle ear regardless of whether the overpressure was induced locally in the middle ear via a transmyringal tube or indirectly by means of an ambient pressure decrease (intact eardrum). The prerequisite for vestibular stimulation seemed to be a certain relation between the middle ear pressure and the ambient pressure. The results of the present study, however, indicate that in actual fact the prerequisite might be a certain relation between the inner ear and the middle ear pressure. This assumption is based on the observation that the NPL was always higher in the recumbent than in the sitting position and in view of the different mechanisms discussed above in connection with the intracranial and the intralabyrinthine pressure, the NPL increase in the recumbent position might be due to an inner ear pressure increase. This assumption is also confirmed by the observation that a still higher NPL was recorded at the examinations in the recumbent position during neck vein compression. However, there is also an increase in arterial pressure in the recumbent position which must be taken into account when discussing the mechanism of A V.

The way in which overpressure in the middle ear affects the vestibular system is still unknown.

Displacement of the inner ear fluid and disturbance of the blood circulation are possible factors. A circulatory disturbance could be due to an increased intralabyrinthine pressure affecting the arterial as well as the venous blood flow. The overpressure in the middle ear might also affect blood vessels which communicate with the inner ear and thus disturb the normal blood flow.

It is known that impulses are regularly emitted by the resting vestibular organ, and a decrease in the discharge in one ear causes a nystagmus directed towards the other, while an increase in the discharge directs the appearing nystagmus towards the affected ear. It has been demonstrated (Ingelstedt et al., 1974) that the nystagmus observed in the experiments of A. V. is always directed towards the ear with the highest FPL. Thus it seems that the overpressure in the middle ear somehow causes an increased discharge of impulses from the affected ear. Such an activation might reflect a genuine stimulation following for example an utriculopetal cupula deviation in the horizontal semicircular canal or be due to a disturbance of the neurophysiological mechanism responsible for the discharge of resting vestibular impulses (pseudo-stimulation).

The results of the present study have indicated a tendency in the vestibular stimulation to increase in intensity with higher FPL values (cf Figs 3 and 4). However, the FPL increase as demonstrated in the figures was induced by a change in posture, and it could not be evaluated to what extent the posture change *per se* might have contributed to the indicated nystagmus increase. From the recordings of subject X, however, different forcing pressure levels were observed in the sitting position at different sessions and as seen from Fig. 5 the nystagmus intensity was higher in the experiments when a higher FPL was recorded. It could not be assessed, however, whether the indicated stronger stimulation was due to a higher  $P_{tm}$  or to a prolonged stimulation time in the examinations where the passive opening of the tube occurred at a later moment during the ascent.

In the present study, pressure asymmetry

between the right and the left ear was observed, but asymmetry *per se* did not require vestibular stimulation unless the restraining pressure in one ear reached a certain level (NPL). Furthermore, the magnitude of asymmetry did not seem to affect the intensity of the vestibular stimulation. The latter is not statistically conclusive, however, and further studies have to be performed.

## ZUSAMMENFASSUNG

Der passive Druckausgleich im Mittelohr bei simulierter Steigungen wurde durch die Körperlage beeinflusst. In liegender Stellung war ein höheres Überdruckniveau im Mittelohr ( $P_{tm}$ ) als in sitzender Stellung um die Eustachischen Tuben zu öffnen.

Personen mit hohem (zwangsmässigen) Öffnungsdruck (FPL) in einem Ohr, die früher alternotisches Schwindel erfahren hatten, wurden simulierten Steigungen ausgesetzt, und zwar in verschiedenen Stellungen.

so regelmässig wie ein geringer, und das bei vestibulärer Stimulation bei einigen Personen. In der Untersuchung im Liegen konnte festgestellt werden, dass der FPL nicht das Niveau des NPL ist. Ein Zusammenhang zwischen dem Druck im Mittelohr und im Innenohr als Voraussetzung für die Stimulation wird diskutiert.

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## ADRENERGIC INNERVATION OF THE NASAL MUCOSA IN CAT

### *A Histological and Physiological Study*

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(Received January 10, 1974)

**Abstract** The adrenergic innervation of the cat nasal mucosa was investigated with fluorescence and electron microscopy. A strong fluorescence attributable to adrenergic nerves was observed on the outer surface of the smooth muscle layer of blood vessels, whereas the fenestrated capillaries showed no evidence of adrenergic innervation. A sparse adrenergic innervation was observed in connection with the mucosal glands. The effects of sympathetic nerve activation and various denervation procedures were evaluated with a local tracer disappearance technique reflecting the vascular events in the exchange vessels of the maxilloturbinal area. Both functional as well as morphological data pointed to an ipsilateral distribution of the sympathetic nerve fibres from the superior cervical ganglion to the nasal mucosa. The sympathetic postganglionic nerve fibres to the nose travel via the tympanic plexus. After passage through the tympanic plexus the major sympathetic nerve supply to maxilloturbinal area travels in the Vidian nerve.

The functional consequences of autonomic nerve activation have been intensively studied in cat nasal mucosa (Malcomson, 1959, Malm, 1973a, b, Änggård & Edwall 1974, Änggård, 1974). However, the majority of the morphological investigations of the autonomic innervation of the nasal mucosa have been performed in human material (Dahlstrom & Fuxe, 1965, Cauna, 1970, Cauna et al, 1972, Ishii, 1970, Ishii & Toriyama, 1972). Thus the interrelationship between morphological findings and functional effects of autonomic nerve activation of the nasal vascular bed has not been the subject of a systematic study in one species.

This investigation has been supported by the Swedish Medical Research Council (Grant no. 40X 3518).

From previous morphological studies it has been assumed that the adrenergic innervation is restricted to blood vessels and that the glands lack an adrenergic innervation (Dahlstrom & Fuxe, 1965, Cauna et al, 1972). This lack of adrenergic innervation of the glands in the cat nasal mucosa is at variance with what is known from other glandular tissues (Eaton, 1968), and could possibly be due to species differences or differences in the techniques used. The autonomic innervation of the nasal glands should therefore be reexamined in the cat with an improved technique (cf. Hokfelt, 1968, Fuxe & Thoenen, 1967).

In patients suffering from 'vasomotor' rhinitis, a Vidian neurectomy has been performed to reduce the parasympathetic activity to the nasal mucosa, thought to be responsible for the symptoms of increased nasal secretion and congestion in this condition (cf. Änggård, 1974). From experimental studies in dogs and cats there is still no agreement as to whether the Vidian nerve contains sympathetic as well as parasympathetic fibres to the nose (Trotter, 1913, Blier, 1930, Malcomson 1959, Jackson & Rooker, 1971, Malm, 1973a). Experimental evidence suggesting a strong sympathetic influence on the nasal vascular bed has been obtained from functional studies in the cat (Änggård & Edwall, 1974). If at Vidian neurectomy both sympathetic as well as parasympathetic fibres are

adrenergic denervation of the nasal mucosa result, with a decrease in the sympathetic innervation tone. In the present study, the adrenergic innervation of the cat nasal mucosa has been examined by light and electron microscopy to provide data for physiological studies in this species. Previous anatomical and morphological results have been obtained after sympathetic denervation, by transection of the superior cervical ganglion, the sympathetic plexus, or the Vidian nerve.

## MATERIAL AND METHODS

Experiments were conducted on 11 cats (15 to 20 months old). In 2 cats only morphological studies were performed.

### Sectioning

Observations were performed under general anesthesia (Nembutal 1 p 30 mg/kg) one to three days before the experiments.

Unilateral sympathectomy was performed in 9 cats by extirpation of the superior cervical ganglion. The tympanic plexus was cut via the middle ear. The tympanic bulla on one side in 2 cats and on both sides in 2 cats. The Vidian nerve was cut through a transorbital approach and transected on one side in 3 cats.

### Disappearance measurements

Five cats were anesthetized with chloralose-anesthesia (50 mg/kg + 100 mg/kg i.v.) and the trachea was cannulated. The pressure in the carotid artery was measured using a Statham pressure transducer (P23A) and recorded on a multi-channel recorder. Rectal temperature was kept constant at 38°C by heating pads. The technique used to measure changes in microcirculation is the same as described in a previous paper (Ånggård & Edwall, 1974). In the absence of vascular events in the exchange vessels are studied by measuring the local disappearance rate of an easily diffusible tracer, from a local depot in the maxilloturbinal area of the nasal mucosa. Radioactivity was

counted for periods of 40 sec. The disappearance rate ( $k$  value), which represents the fractional elimination of the depot per minute, was calculated on a computer for each time interval. When sympathetic stimulation was performed the cervical sympathetic nerve was dissected free from the vagal nerve and transected. The nerve was stimulated in the cranial direction using a bipolar silver electrode. The stimuli were monophasic square wave pulses (6 V, 1 msec) delivered by a Grass model S4 stimulator. Stimulation frequencies were varied in random order.

### Fluorescence microscopy

After the functional studies the anesthetized animal was decapitated and pieces of the nasal mucosa were taken from the septum, the maxilloturbinal, ethmoturbinal and nasoturbinal area. The specimens were freeze-dried and treated with formaldehyde gas for the demonstration of biogenic amines according to the histochemical method of Falck & Hillarp (for ref. see Corrodi & Jonsson, 1967). A Zeiss fluorescence microscope with a dark field condenser was used. Photomicrographs were taken using Scopex film.

### Electron microscopy

In order to facilitate the demonstration of sympathetic adrenergic nerve terminals by electron microscopy, two cats were pretreated with 5-hydroxydopamine (3,4,5-trihydroxyphenylethylamine hydrochloride)  $4 \times 20$  mg/kg i.p. over a period of 48 hours (Tranzer & Thoenen, 1967). Before decapitation the animals were anesthetized with Nembutal (30 mg/kg i.p.).

Thin slices from the nasal mucosa were fixed in ice-cold 3% potassium permanganate in 0.1 M sodium phosphate buffer at pH 7.0 for 45 minutes (Richardson, 1966; Hokfelt, 1968). After rinsing in Ringer solution, the specimens were dehydrated in ethanol and embedded in Epon (Luft, 1961). Ultrathin sections were cut with a diamond knife on an LKB ultratome. The sections were briefly contrast-stained in lead citrate (Reynolds, 1963). Electron microscopy was performed using a Siemens Elmiskop I.

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### *Sectioning*

Sympathetic denervations were performed under general anesthesia (Nembutal 1 p 30 mg/kg) one to three days before the experiments. Bilateral sympathectomy was performed in 2 cats by extirpation of the superior cervical ganglion. The tympanic plexus was cut via the middle ear. In 2 cats the tympanic bulla on one side in 2 cats and on both sides in 2 cats. The Vidian nerve was transected through a transorbital approach and transected on one side in 3 cats.

### *Tracer disappearance measurements*

All cats were anesthetized with chloralose-anesthesia (50 mg/kg + 100 mg/kg i.v.) and the nasal cavity was cannulated. The pressure in the superior nasal artery was measured using a Statham pressure transducer (P23A) and recorded on a Beckman multichannel recorder. Rectal temperature was kept constant at 38°C by heating pads. The technique used to measure changes in microcirculation is the same as described in our previous paper (Ånggård & Edwall, 1974). In the present study, vascular events in the exchange of substances are studied by measuring the local disappearance rate of an easily diffusible tracer, from a local depot in the maxilloturbinal area of the nasal mucosa. Radioactivity was

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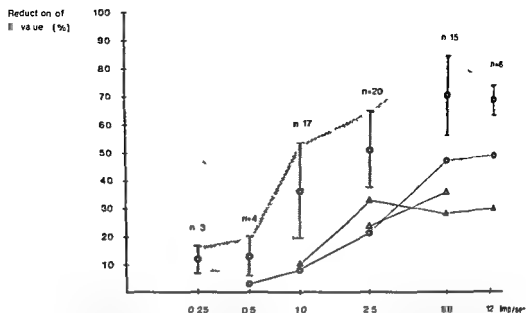


Fig 1 The influence of sympathetic nerve stimulation on tracer disappearance rate after Vidian neurectomy (3 cats). The shaded curve represents the normal responses

found in a previous study of non-denervated cats) (Änggård & Edwall 1974)

## RESULTS

### Tracer disappearance measurements

Upon stimulation of the ipsilateral sympathetic nerve, the tracer disappearance rate on the non-denervated side of the cat was reduced to the same degree (Fig 1) as was found in a previous study (Änggård & Edwall, 1974). After a bilateral chronic sympathectomy no reduction in tracer disappearance rate was seen on stimulation of the contralateral sympathetic nerve. In 3 out of 11 cats in which the tympanic plexus had been cut a complete transection was achieved. In these animals no response was recorded following nerve stimulation.

After transection of the Vidian nerve in 3 cats, stimulation of the ipsilateral cervical sympathetic nerve resulted in reduced responses which were in all cases significantly smaller than the normal sympathetic responses (Fig 1).

### Fluorescence microscopy

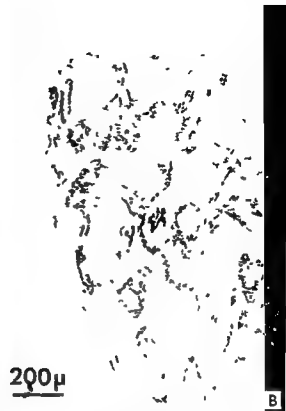
In all parts of the normal nasal mucosa investigated there was a rich fluorescence typical of sympathetic adrenergic nerves. Blood vessels of various sizes were surrounded by a large number

of adrenergic nerves (Fig 2A, B). Fluorescence was particularly prominent around the arterial and venous sinusoids. In some cases varicosities could not be distinguished from the heavy innervation. Below the small blood vessels, evidently arterioles, were seen surrounded by an adrenergic nerve. In this region some adrenergic nerves seemed to end freely just below the basement membrane. The nerve fibres did not penetrate the membrane and no fluorescence was seen inside the epithelium. In the glandular mucosa a sparse network of nerve fibres was seen to surround the septa (Fig 2A).

Unilateral cervical sympathectomy resulted in a complete ipsilateral disappearance of fluorescent fibres (Fig 3) while fluorescence remained on the contralateral side. When the tympanic plexus was cut a complete absence of fluorescence was seen on the operated side. A large accumulation of fluorescent fibres at the proximal end of the cut tympanic plexus was observed. Some fluorescence was observed even at the distal end. In 1 of 3 cats the tympanic plexus was found to be intact



The adrenergic innervation in the nasal mucosa (maxilloturbinal area). Fluorescence is most apparent around arteries and sinusoids. A sparse network of



fluorescent nerve fibres is observed around the glands (A). (B) Ethmoturbinal area. Fluorescence not as prominent as in the maxilloturbinal area.

ted. In such cases a normal fluorescence was seen, as well as a normal response on tracer appearance rate on sympathetic stimulation. In all cats with Vidian nerve transection a reduced and scattered fluorescence was observed in the maxilloturbinal mucosa. The remaining fluorescence was most apparent around the arteries (Fig. 4). In the nasoturbinal and ethmoturbinal area, fluorescence was unaffected.

#### *Electron microscopy*

Adrenergic nerve terminals were easily recognized with the technique employed. Apart from mitochondria, the terminals contained a varying number of dense cored vesicles with a diameter of about 500 Å and a few large granulated vesicles with a diameter of about 1 000 Å. Nerve terminals containing exclusively small non granulated vesicles were seen. They had the appearance of

cholinergic terminals, as described by Grillo (1966) and will be referred to as cholinergic.

In the mucosa mostly unmyelinated bundles of nerve fibres were seen. These unmyelinated axons were located in groups and enfolded in a Schwann cell. In these bundles it was difficult to distinguish between adrenergic and cholinergic nerve fibres, since many fibres contained no vesicles. The extensive adrenergic innervation of the blood vessels was revealed by the presence of terminals containing granular vesicles. Rows of adrenergic nerve terminals were located on the outside of the smooth muscle layer around the arteries, arterioles, and sinusoids (Fig. 5). The terminals were partly enfolded in a Schwann cell, with their free surfaces facing the smooth muscle fibres. Scattered cholinergic terminals containing agranular vesicles were regularly observed together with adrenergic terminals.

They were enfolded by the same Schwann cell (Fig. 6). Fenestrated capillaries were present under the respiratory epithelium and around the mucosal glands. Neither cholinergic nor adrenergic innervation could be demonstrated around these vessels.

The principal innervation of the blood vessels was adrenergic in nature, whereas the majority of nerve terminals in the innervation of the nasal glands contained small agranular vesicles, considered to be cholinergic. Bundles of nerve fibres and terminals were seen among the nasal glands and in interacinar spaces. The axons were partly or completely surrounded by a Schwann cell. Most terminals contained agranular vesicles, but in almost every group of terminals there were one or two containing dense cored vesicles considered to be adrenergic in nature (Fig. 7). In the glandular parts the cholinergic nerve terminals outnumbered the adrenergic terminals by



roughly 10 to 1, while the reverse was found around the blood vessels.

## DISCUSSION

In the present investigation techniques the function as well as the morphology of adrenergic innervation of the cat nasal were used.

The present tracer disappearance has previously been used to study effects of sympathetic and parasympathetic nerve activity in the cat nasal mucosa. The technique reflects the vascular events in the turbinate area of the nasal mucosa (cf. Edwall 1974, Änggård 1974). The histochemical fluorescence technique (cf. Falck & Hillarp (for ref. see Corrodi & 1967) provides a possibility of visualizing

Fig. 3. Nasal septum after an ipsilateral cervical sympathectomy. No fluorescence observed.



Electron microscopic picture of an artery in the mucosa. Adrenergic nerve terminals (A) located along the smooth muscle fibres (SM). Lumen (L). Cholinergic terminal (arrow)  $\times 6400$





Fig. 6 Higher magnification of framed area in Fig. 5, illustrating adrenergic (A) and cholinergic (C) nerve terminals along a smooth muscle fibre (M)  $\times 22\,750$

ic neurons and thus demonstrating the effects of sympathetic denervation. The specificity of the technique and the fact that the fluorescence completely disappeared following superior cervical ganglionectomy suggested that the fluorescence in the nasal mucosa represents postganglionic sympathetic nerve fibres.

Postganglionic transection results in a degeneration of the distal nerve axons and resulted in the abolition of fluorescence. Transection of the tympanic plexus thus resulted in a complete ipsilateral sympathectomy and accumulation of transmitter in the proximal end of the cut nerve, indicating that the postganglionic sympathetic nerve fibres constitute a part of the tympanic plexus in the cat. This is at variance with what is assumed to be the case in man, where the postganglionic fibres are considered to form an internal carotid plexus and then, as the deep petrosal nerve, join the parasympathetic fibres

in the Vidian nerve to continue further nasal mucosa (Ritter, 1970). However, an anatomical difference might be explained by embryonic regression of the internal carotid artery as occurs in the cat, where the adult cat is replaced by a fibrous (Walker, 1972).

The conflicting reports on the sympathetic nerve content of the Vidian nerve (Baker, 1966; Malcolmson, 1959; Jackson & Rooker, 1973a; Malm, 1973a) were suggested by Jackson & Rooker to be due to a spread of the current to surrounding tissues on Vidian stimulation. With the present histofluorescence technique it could be clearly demonstrated that the maxilloturbinal area in the cat is sympathetically innervated via the Vidian nerve, while nasoturbinal and ethmoturbinal areas are innervated along other pathways. After a neurectomy the fluorescence was sparse



7 Electron microscopic picture of the glandular part of the nasal mucosa. Nerve fascicle between

glandular cells (Gc) contains adrenergic (A) and cholinergic (C) nerve terminals  $\times 32\,500$

maxilloturbinal vascular bed and sympathetic stimulation resulted in only slight reductions in re-exchange rate. This indicates that remaining sympathetic innervation affected exchange vessels in the maxilloturbinal area, though to a lesser degree.

In a rhinomanometric study of the cat, Malm (1963a) found an increase in nasal patency when stimulating the contralateral sympathetic nerve, at variance with the results of the present

study where no fluorescent fibres were seen to cross the midline and no effect was seen on the exchange vessels on contralateral sympathetic stimulation. However, if a collateral circulation does exist, a leakage of the adrenergic transmitter to the blood stream (cf. Fuxe & Sedvall, 1965) might explain this variance in results. As a relative difference exists in the degree of the vasoconstrictor response between the exchange and capacitance vessels in the nasal mucosa



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7 Electron microscopic picture of the glandular cells (Gc) of the nasal mucosa. Nerve fascicle between

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(Änggård & Edwall, 1974), a vasoconstrictor response would most likely appear in rhinomanometric measurements, which mainly reflect the capacitance function of the nasal mucosa.

Previous morphological studies on the nasal mucosa in man and various experimental animals have failed to demonstrate an adrenergic innervation of the glands (Cauna et al., 1972; Dahlström & Fuxe, 1965). In the present study on the cat, however, it was observed that, apart from blood vessel innervation, there is also a sparse adrenergic innervation around the acini in the mucosal glands. This variance in results can probably be ascribed to the use of different techniques for the demonstration of biogenic amines. The present ultrastructural technique used permanganate as a fixative, as suggested by Richardson (1966) and further developed by Hokfelt (1968). Furthermore, pretreatment of the cat with the 'false' transmitter 5-hydroxydopamine was used to increase the levels of amines in the adrenergic storage vesicles (Tranzer & Thoenen, 1967), thus facilitating the identification of adrenergic neurons. Thus almost all large and small vesicles in adrenergic terminals contained a dense core. In the glandular parts the majority of nerve fibres appeared empty or contained agranular vesicles, described by Grillo (1966) as cholinergic. However, nerve fascicles around and in between the acini contained nerve terminals with dense-cored vesicles as evidence for an adrenergic innervation as well.

The autonomic dual innervation of the nasal glands corresponds to what is known from salivary glands (cf. Emmelin, 1968; Norberg et al., 1969). This similarity between salivary glands and the nasal glands might indicate that the physiological aspects of autonomic nerve activation might be similar as well, though the sparse adrenergic innervation suggests that cholinergic effects may dominate the secretory responses within the nasal mucosa.

#### ACKNOWLEDGEMENTS

Skilful technical assistance was given by Mrs Sonja Bjornberg and Mrs Marie Louise Spångberg.

#### ZUSAMMENFASSUNG

Die adrenerge Innervation der Nasenschleimhaut der Katze wurde Fluoreszenz- und elektronenmikroskopisch untersucht. Eine kräftige Fluoreszenz, hervorgerufen durch adrenerge Nerven, wurde an der inneren flachen der glatten Muskelschicht der Blutgefäßwand festgestellt, während die gefenesterten Kapillaren keine Fluoreszenz zeigten.

Verschiedener Denervationsmassnahmen wurde Hilfe einer lokalen Tracer-Abkling Technik angewendet, welche die vaskulären Auswirkungen in den Kapillaren der Maxillotubinalregion widerspiegelt. Funktionelle als auch morphologische Ergebnisse auf eine ipsilaterale Verteilung der sympathischen Fasern vom Ganglion cervicale superior hinweisen. Die sympathischen postganglionären Nervenfasern laufen über den Plexus tympanicus zur Nasenschleimhaut. Der Durchtritt durch den Plexus tympanicus ist der Hauptanteil der sympathischen Nervenfasern in der Maxillotubinalregion auf dem Wege der Nerven.

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$\beta$ -ADRENERGIC RECEPTORS IN THE VESSELS OF THE CAT NASAL MUCOSA

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(Received February 7, 1974)

**Abstract** The effects of two close arterially given  $\beta$  receptor stimulating agents, isoprenaline and terbutaline, on resistance and capacitance vessels in the nasal mucosa of the cat were investigated in terms of measurements of mucosal venous blood flow and changes in nasal patency, respectively. Both drugs decreased blood flow resistance (perfusion pressure/venous blood flow) and nasal patency, i.e. dilated resistance and capacitance vessels. In equi weight doses isoprenaline was more effective than terbutaline in decreasing the blood flow resistance and the nasal patency. The  $\beta$  receptor blocking agent propranolol reduced the effect of isoprenaline and terbutaline on the resistance vessels and caused a parallel shift of the semi logarithmic dose-response curves of both drugs. It is concluded that the effects of isoprenaline and terbutaline in the nasal vascular bed of the cat are mediated via  $\beta$  adrenergic receptors at least in the resistance vessels.

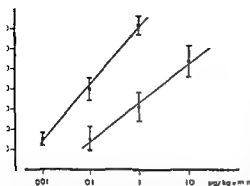
effects of two  $\beta$  receptor stimulating agents, isoprenaline and terbutaline, were studied before and after propranolol administration.

## METHOD

The observations were made on cats, anaesthetized with  $\alpha$ -chloralose (about 80-120 mg/kg) after induction with ether. The animals were tracheotomized. Venous blood flow and changes in nasal patency were recorded from the nasal cavity using methods which have been described previously (Malm, 1973b, 1974). In brief, the vein leaving the nasal mucosa at the pterygopalatine foramen was cannulated for blood flow recording. For this purpose, venous blood flow from the nasal vein was diverted through an optical blood flow recording unit and then returned to the animal via a cannula connected to the right jugular vein. The blood flow was continuously recorded on a moving paper. The height of the venous outflow water was set to 6 to 8 cm above the pterygopalatine foramen to obtain about normal venous outflow pressure (Malm, 1974). Changes in nasal patency were estimated in terms of changes in a thin walled water-filled balloon placed in the right nasal cavity. The balloon was connected via a polyethylene tubing to an electronic pressure transducer (EMT 31, E. Schöndander) for recording on a polygraph (M81, Elema-Schöndander). In the control state the pressure in the balloon was

and Jackson (1968) studied the behavior of nasal vessels in terms of changes in nasal patency in the dog in response to  $\beta$  receptor stimulating and  $\beta$  receptor blocking agents. They concluded that the nasal vessels of the dog lack  $\beta$  adrenergic receptors. In an investigation on cats (Malm, 1973a), in which both the nasal venous blood flow and the nasal patency were studied, the dilator effects of isoprenaline seemed to be reduced by the  $\beta$  receptor blocking agent propranolol. Although the number of observations in that study was small, the results suggested the existence of  $\beta$  receptors in the cat nose vessels. The present investigation is a more thorough study of this problem, in which the

The present study was supported by grants from C. G. Lundberg's Foundation and the Swedish Medical Research Council (B74-14X 2210-08C).



Mean changes ( $\pm$  S.E.M.) of nasal blood flow resistance after 1 min infusions of different doses of isoprenaline (x) and terbutaline (o) in 7 cats. The ordinates are the decrease of blood flow resistance as a percentage of control resistance in a linear scale and the abscissae are the doses of the drugs in a logarithmic scale. Regression lines are calculated from the mean changes by the least squares method ( $p < 0.01$  for both correlations).

of  $H_2O$ . Arterial blood pressure was recorded from the right femoral artery via a mercurianometer on the kymograph. Close arterial infusions were given through the right lingual artery in retrograde direction via a polyethylene catheter and with the aid of a constant infusion pump. Venous injections were given in the right jugular vein. Just before the insertion of the catheters the cats were heparinized. The cervical vagus nerve was acutely cut on the right side before the observations were made. Vascular resistance was calculated by dividing the perfusion pressure by the venous blood flow. The responses of the resistance vessels in the nasal mucosa to the drugs were assessed as the per cent change of vascular resistance from the control value prevailing before drug administration. Pressure changes in the balloon have been shown to be linearly related to changes of nasal patency and can be used as a semi quantitative measure of evoked changes in the capacitance vessels in the nasal mucosa (Malm, 1974). The capacitance responses were expressed in terms of pressure changes in the balloon (cm  $H_2O$ ).

In the following drugs were given isoprenaline hydrochloride, terbutaline sulphate, propranolol hydrochloride and papaverine sulphate.

Paired comparisons and Student's  $t$  test were used in the statistical analyses.

## RESULT

Isoprenaline and terbutaline were given in a series of 7 cats close arterially to the nasal mucosa as infusions of as a rule 1 min duration. Four doses of each drug were generally administered: Isoprenaline 0.001, 0.01, 0.1 and 1  $\mu$ g/kg b.w.  $\times$  min and terbutaline 0.01, 0.1, 1 and 10  $\mu$ g/kg b.w.  $\times$  min. The lowest dose of the two drugs was nearly always subthreshold. In suprathreshold doses the two drugs always evoked an increased venous flow and a decreased nasal patency and in response to the highest dose of each drug often a detectable decrease of arterial blood pressure. The responses of the resistance vessels were expressed as the percentage decrease of blood flow resistance below the control level and the results are summarized in Fig. 1, which gives mean values  $\pm$  S.E.M. The difference between the vascular resistance changes evoked by equi-weight doses of isoprenaline and terbutaline was significant ( $p < 0.001$ ). Regression analyses (the least squares method) showed that the dose-response curves were linear ( $p < 0.01$  for both correlation coefficients) in semi logarithmic plotting. From these two regression lines the dosages of the two drugs required to evoke the same blood flow resistance change may be deduced. The relative effectiveness of the two drugs on a weight basis can thereby be estimated. For example, to evoke a 20% decrease of vascular resistance, the dose had to be 13 times larger for terbutaline than isoprenaline.

The effects of these two drugs on the nasal capacitance vessels were also studied with the balloon technique. The balloon pressure always increased, indicating a dilatation of the capacitance vessels, after suprathreshold doses of the two drugs. The effects of both drugs were relatively small. On an equi-weight basis, however, isoprenaline was more effective than terbutaline, the difference being significant, but only at the  $p < 0.05$  level. The dose-response curves were



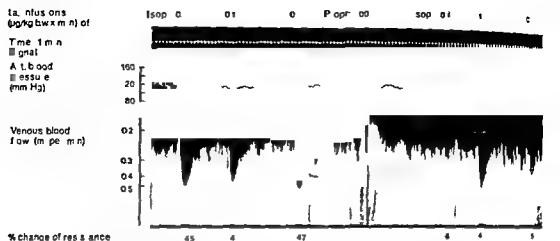


Fig 2 Cat 25 kg Effects of isoprenaline (Isopr) and propranolol (Propr) on arterial blood pressure and nasal venous blood flow. Infusions are marked below the time record.

not linear in semi logarithmic plotting ( $p > 0.05$  for the correlation coefficients) why no data on the relative effectiveness of the two drugs in changing nasal balloon pressure can be given.

These results evoked by two  $\beta$  receptor stimulating agents suggest the presence of  $\beta$  adrenergic receptors in nasal resistance and capacitance vessels. The problem was therefore further investigated with the aid of the  $\beta$  receptor blocking agent propranolol administered close arterially (4 cats) to the nasal mucosa as well as intravenously (6 cats).

The magnitude of the venous blood flow increase evoked by isoprenaline and terbutaline was found to be reduced by propranolol and an example of this can be seen in Fig 2. In this cat three repetitive infusions of isoprenaline (0.1  $\mu\text{g/kg b.w.} \times \text{min}$  intra arterially) elicited quite similar increases of venous blood flow (dilations of the resistance vessels) before propranolol administration. After propranolol (100  $\mu\text{g/kg b.w.} \times \text{min}$  for 3 min intra arterially) isoprenaline in the same dose as mentioned evoked a much smaller increase of blood flow. An about tenfold increase of the dose of isoprenaline was now required to produce as large dilator effects of the resistance vessels as before propranolol. The calculated decreases of the flow resistance are also given in Fig 2 below the tracings.

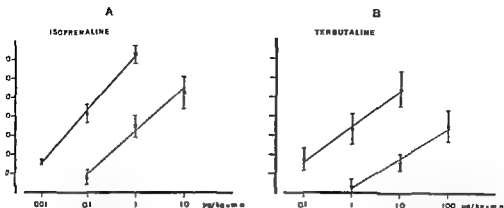
The bulk of data in this series of experiments was obtained after intravenous administration

of propranolol in a dose of 1.3 mg/kg. A pronounced arterial blood pressure fall ensued upon the intravenous injection of propranolol. Isoprenaline and terbutaline were never not given until the blood pressure returned to near normal values. The results of these experiments are summarized in Table 1 (mean values  $\pm$  S.E.M.). Panel A refers to isoprenaline and panel B to terbutaline. The solid lines passing through the mean effects in the resistance vessels before and after propranolol administration. It can be seen that propranolol caused a parallel shift of the dose-response curve to the right. The difference for equivalent dose was significant at the  $p < 0.001$  level for isoprenaline and at the  $p < 0.02$  level for terbutaline.

The capacitance responses (balloon pressure increases) evoked by terbutaline were significantly reduced by propranolol ( $p < 0.05$ ), while those evoked by isoprenaline were not significantly affected ( $p > 0.05$ ).

These studies with the  $\beta$  receptor blocking substance propranolol further support the idea that  $\beta$  adrenergic receptors exist in the submucosa of the nasal mucosa, at least in the resistance vessels.

The possibility remains that propranolol, in addition to its  $\beta$  receptor blocking effect, has a non specific action on the resistance vessels. In an attempt to elucidate this problem



Mean changes ( $\pm$  S.E.M.) of nasal blood flow re-  
sponse in per cent of control after i.v. infusions of differ-  
ent doses of isoprenaline (panel A) and terbutaline (panel  
B) (—) and after (----) propranolol 1–3 mg/kg

in 6 cats. Ordinates and abscissa are as in Fig. 1.  
Regression lines are calculated from the mean changes  
by the least squares method ( $p < 0.02$  for all four correla-  
tion coefficients).

ive study (three cats) was performed with  
er dilator substance, papaverine, which has  
receptor stimulating effect. Isoprenaline and  
verine were infused in doses which evoked  
variable dilatations of the resistance vessels  
re control state. After propranolol adminis-  
tration papaverine was about equally effective  
dilating the resistance vessels in contrast to  
effect of isoprenaline which was clearly  
reduced.

## DISCUSSION

The present investigation shows that the two  
receptor stimulating substances, isoprenaline  
and terbutaline, dilate the resistance vessels in the  
nasal mucosa of the cat. The magnitudes of these  
dilatations were clearly reduced by propranolol.  
Furthermore, propranolol seemed to cause a  
parallel shift to the right of the lines, which in  
logarithmic plotting represented the dose-  
response curves of isoprenaline and terbutaline.  
These parallel shifts may suggest a  
competitive antagonism. The results thus speak  
in favour of the existence of  $\beta$ -adrenergic recep-  
tors in the resistance vessels, this concept was  
further supported by control experiments with  
the dilator agent papaverine.  
The two  $\beta$  receptor stimulating substances al-  
so dilated the capacitance vessels. Propranolol  
failed to reduce the magnitudes of the capaci-

tance responses to the two drugs, but in this  
material the reduction was statistically signifi-  
cant only for terbutaline. It thus appears that  
 $\beta$  adrenergic receptors are present also in the  
nasal capacitance vessels, although perhaps less  
abundantly than in the resistance vessels. Such  
an uneven distribution of the  $\beta$  receptors has  
previously been discussed for the vascular bed  
of skeletal muscle (Mellander, 1960, Johnsson  
& Öberg, 1968).

Hall & Jackson (1968) could not selectively  
block the responses to isoprenaline on the nasal  
patency in dogs with propranolol and therefore  
they suggested a lack of  $\beta$ -adrenergic receptors  
in the nasal vessels. With their method, however,  
the effects were only observed in the capacitance  
vessels and their conclusion thus has to be re-  
stricted to events in this type of vessel in the  
dog. The present results clearly indicate the  
presence of  $\beta$ -adrenergic receptors in the nasal  
vessels but, as mentioned, perhaps mainly in the  
resistance vessels. Of course it cannot be ex-  
cluded that species differences exist between the  
dog and the cat.

Lands et al. (1967) have classified  $\beta$  adrenergic  
receptors in different groups designated  $\beta_1$  and  
 $\beta_2$ . According to their concept, heart muscle and  
intestinal smooth muscle contain  $\beta_1$  receptors  
and smooth muscle of the respiratory tract,  
blood vessels, uterus and diaphragm  $\beta_2$ -recep-

tors Isoprenaline stimulates both  $\beta_1$  and  $\beta_2$ -receptors, terbutaline affects mainly  $\beta_2$ -receptors (Persson & Olsson, 1970). These latter authors found that isoprenaline in equi-weight doses was as much as 200 times more effective than terbutaline in increasing the contractile force by 20% in heart muscle of the cat ( $\beta_1$ -receptor effect), whereas it was only 20 times more effective in reducing peripheral blood flow resistance in the cat hind limb or lower half of the body ( $\beta_2$ -receptor effect). Since isoprenaline in this study was 13 times more effective than terbutaline in reducing nasal blood flow resistance by 20%, it appears that these  $\beta$ -receptors belong to the  $\beta_2$ -type. It should be stressed, however, that the classification in  $\beta_1$ - and  $\beta_2$ -receptors has been criticized in recent years. Thus, Brittain et al (1970) consider it not necessary to postulate a diversity of  $\beta$  receptors as they do not vary greatly in their sensitivity to the natural catecholamines noradrenaline and adrenaline.

Isoprenaline and terbutaline are drugs used in man, and especially terbutaline has a beneficial effect in bronchial asthma. The question whether therapeutical doses of the two drugs significantly could affect the human nasal blood flow and patency may be raised in view of the present results and should be elucidated by future investigations.

### ACKNOWLEDGEMENTS

Generous supply of terbutaline sulphate from Draco AB, Lund, Sweden, is gratefully acknowledged.

### ZUSAMMENFASSUNG

Der Effekt von zwei  $\beta$  Rezeptor stimulierenden Substanzen, Isoprenalin und Terbutalin auf die Resistanz und Kapazitanzblutgefäße in der Nasenschleimhaut von Katzen wurde durch Messung der venösen Durchblutung in der Nasenschleimhaut und Veränderungen im Nasenraum untersucht. Beide Substanzen, in der Nähe der Nase arteriell infundiert, verminderten den Widerstand der

Durchblutung und verursachten eine Abnahme der Nasenschleimhaut, die Substanzen erwiesen sich als effektiver, wenn beide Substanzen in gleichen Mengen gegeben wurden. Die  $\beta$  blockierende Substanz Propranolol reduzierte den Effekt von Isoprenalin.

Terbutalin im nasalen vaskulären Bett der Katze vermindert in den Resistanzblutgefäßen, via  $\beta_2$ -Rezeptoren vermittelt wird.

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## MUCOCILIARY WAVE PATTERN

### *An analysis of surface light reflections*

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(Received January 14, 1974)

*Abstract* Experiments were performed regarding surface reflections obtained from the rabbit trachea in vitro a standardized method using a microscope photographic technique. Comparative experiments have been conducted on a water surface with induced surface waves. The result of these studies revealed that the surface reflections obtained from mucous membranes of the respiratory tract are composed of at least two components, i.e. from the mucous surface and from the cilia beneath the surface.

Ciliary activity of the respiratory epithelium can be examined indirectly via variations of surface reflections recorded from the illuminated mucous membrane surface (Mercke et al, 1974). Indirectly, by the use of intracellular electrodes (Håkansson & Toremalm, 1966). Intracellular recordings are valuable from a theoretical point of view, but cannot be used for longterm experiments. Indirect methods are therefore best suited for applied experimental research. The cilia are intimately associated with the medium in which they work, therefore, the chemical and physical properties of the mucous layer are of great importance. The light reflections may arise from the mucous layer and from the surface of the cilia beneath the surface. The ciliary activity can be studied via surface light reflections should be correctly described as *mucociliary wave frequency* rather than as *ciliary beat frequency*, the term currently utilized.

This investigation has been supported by grants from the Swedish Medical Research Council, Project number 14X 3897-01

The object of the present research has been to investigate and compare surface reflections from mucous waves induced by mucociliary activity of the rabbit trachea in in vitro experiments and surface reflections initiated by a vibrating tone probe on a water surface in order to analyse the origin of reflections. The following questions are considered

- 1 Do the recorded light reflections appear in the mucous layer alone?
- 2 Do the light reflections appear from the surface of cilia?
- 3 Do the reflections have a double origin?

## METHODS

### *Recording of mucociliary waves*

The mucociliary activity on the surface of rabbit trachea has been recorded by an indirect light reflection method for in vitro experiments as described in detail in a previous paper (Mercke et al, 1974). A light beam was aimed at the inner surface of a rabbit trachea mounted in an experimental chamber. Variations in light surface reflection brought about by movements of the cilia in the covering mucous layer were focused in a binocular microscope. The optical signals were converted to electrical pulses by a photomultiplier attached to one of the ocular tubes. The pulses were magnified and interfering frequencies e.g. from the mains were eliminated by

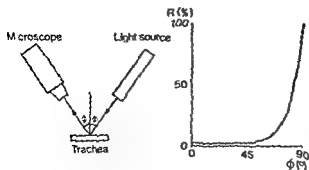


Fig. 2 The relation between the angle of incidence and the relative intensity of the reflected light.

a clipping filter. The light reflection variations were instantaneously recorded.

Recordings were also made from the trachea through a  $12\ \mu$  thick transparent polythene plastic film and a  $150\ \mu$  thick piece of glass, respectively. The mucous layer was covered in this way in order to distinguish between reflections from the true mucous waves of the secretion layer and reflections obtained from the waves of sweeping cilia just below the surface.

In all experiments the temperature and relative humidity parameters were kept constant ( $38^\circ\text{C}$ , r.h. 90–100%).

#### Recording of artificial waves

A tone probe connected to a tone generator (Hewlett Packard 202 C) was submerged in ordinary tap water in a plexiglass case. Waves with a frequency of 10, 15 or 20 Hz were induced on the water surface. The wave patterns were picked up with the method described above. The tone generator output was recorded simulta-

neously on an oscilloscope screen by comparison.

The waves induced on the water surface were also in this case investigated when a  $12\ \mu$  transparent polythene plastic film or a thick piece of glass was kept floating by tension forces in front of the microscope. Recordings of light reflections were through each of these covering materials.

#### Theoretical calculations regarding surface reflections and wave movements

The experimental design is illustrated to the right in Fig. 1. Varying intensities of reflection are obtained according to corresponding variations of the angle between the incident light and the reflected light.

The relationship between the intensity of reflected light ( $R$ ) and the angle of incidence ( $\phi$ ) is illustrated in the diagram shown to the right in Fig. 1. It is presumed that the amplitude of the secretional waves are small as compared with the wave length (Barnett & Munn 1971). Therefore the angle of incidence varies a little. From this it is clear that the light intensity variations also are reduced due to the slight slope of the curve  $R(\phi)$  when  $\phi < 45^\circ$ .

Since the light is directed against a surface of secretions, the reflection takes place on a curved surface. Therefore, the reflected light is either converged or diverged depending on the configuration of the surface. A concave and a convex reflecting surface is illustrated in Fig. 2.

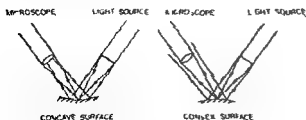


Fig. 2 Light reflections from a wave trough (left) and a wave ridge (right).

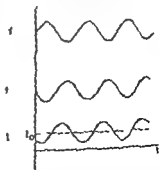


Fig. 3 The relation between wave patterns and intensity  $I$ . Ordinary sinus wave  $f$ , second derived sinus wave  $f''$ , theoretically deduced light intensity  $I$ , basic light intensity,  $t$ , time.

presume that the mucous wave is approxi-  
by the formula

$$\xi_0 \sin \omega \left( t - \frac{x}{r} \right)$$

$x$  position coordinate,  $t$ =time,  $\xi_0$ =  
amplitude,  $\omega$ =angle velocity, and  $r$ =velocity of  
wave. The degree of curvature of a mathe-  
matical function can be expressed by its second  
derivative. The second derivative of the function  
is obtained as

$$f''(x, t) = -\xi_0 \frac{\omega^2}{r^2} \sin \omega \left( t - \frac{x}{r} \right)$$

Assume that the recorded intensity varies  
proportionally to

$$I = \text{constant} \int_{x-\Delta x}^{x+\Delta x} f''(x, t) dx$$

$I$  recorded intensity,  $I_0$ =basic light  
intensity, and  $\Delta x$ =the side of a square cor-  
responding to the inspected area in the micro-  
scope. In reality the area is circular, but the  
difference is of little practical import-  
ance. The following formula is obtained after  
integration and simplification

$$I = \text{constant} \sin \omega \left( t - \frac{x}{r} - \alpha \right) \sin \beta$$

$\beta > 0$  for  $\Delta x < \frac{\lambda}{2}$  where  $\lambda$  wavelength

Thus we get the same periodicity of the  
reflection as of the secretion wave. This  
is seen in Fig 3

## RESULTS

Recordings of mucociliary wave patterns from  
rabbit trachea are seen in Fig 4. For routine

...

Fig 4 Mucociliary wave patterns from the rabbit  
trachea. Recording speed 25 mm/sec (above) and  
50 mm/sec (below). Frequency 12 waves/sec

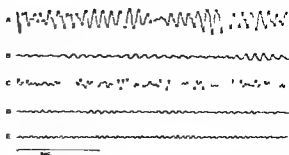


Fig 5 Mucociliary wave patterns from the rabbit trachea. A, Typical frequency and amplitude shifts. Mean frequency 12 waves/sec. B, Recording from the same place with a plastic film covering the area of observation. Mean frequency 15 waves/sec. C, Recording from an untouched trachea. Mean frequency 19 waves/sec. D, Recording through a glass cover placed on the same place of the tracheal surface. Mean frequency 15 waves/sec. E, Recording through the glass cover after 15 min. Mean frequency 17 waves/sec.

purposes a low recording speed of 25 mm/sec is used. For frequency determination and for documentation the speed is raised to 100 mm/sec. Amplitude and frequency variations are easily identified. The mean frequency calculated during 30 sec on this record is 12 waves/sec.

Another record from the rabbit trachea is shown in Fig 5A. Typical amplitude and frequency shifts are seen. The frequency is 11–15 waves/sec (mean value 12 waves/sec). Fig 5B shows the effect when a small transparent plastic film is placed on the tracheal surface in front of the microscope. The amplitudes of the light reflections are decreased and the wave frequency slightly increased (mean value 15 waves/sec).

In the next experiment the initial mean frequency was 19 waves/sec (Fig 5C). A piece of glass measuring 15 × 15 mm and 0.15 mm thick, weighing about 0.1 mg, was placed on the tracheal surface. The amplitudes of the light reflections were reduced. There was also a slight fall in frequency (mean value 15 waves/sec as seen in Fig 5D). After about 15 min with the glass cover in place the amplitudes were still small but the frequency was somewhat increased (Fig 5E).

Simultaneous recordings from the tone gene-

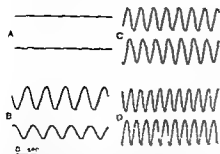


Fig 6 Simultaneous recordings from the tone generator output (above in A, B, C and D) and water surface waves recorded by the photomultiplier method (below). A: Tone generator off; B: 10 Hz; C: 15 Hz; D: 20 Hz

rator output and the reflections picked up by the described method from waves induced on a water surface are demonstrated in Fig 6. The upper oscillograms in A, B, C and D are obtained from the tone generator and the recordings below from the photomultiplier. In Fig 6A the tone generator is off; B, C and D illustrate comparisons at 10, 15 and 20 Hz respectively.

The effect observed when a thin plastic film or a piece of glass is placed on the water surface is demonstrated in Fig 7. Three different frequencies of 10, 15 and 20 Hz have been used. The recordings above are obtained directly from the tone generator output, those below from surface reflections via the photomultiplier. In the experiments shown to the left a polythene plastic

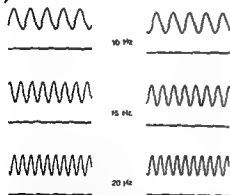


Fig 7 Simultaneous recordings from the tone generator output and water surface waves recorded by the photomultiplier method. To the left: experiments with plastic film covering the area of registration. To the right: the same procedure with a thin glass covering. Three frequencies (10, 15 and 20 Hz) are used. The recordings above are from the tone generator output and the recordings below are from the photomultiplier.

film covering is placed on the water surface, reducing the waves. In the recording to the right the water waves are eliminated by a piece of glass.

## DISCUSSION

During the last four decades the ciliary transport of the respiratory tract has been intensively studied with the use of objective methods. Much interest has previously been focused on the transport velocity, the ciliary beat frequency and the dynamics of ciliary movements (Proetz, 1953; Rivera, 1962). More recently, the medium in which the cilia function has attracted increasing attention (Barnett & Møller, 1970; Sade et al., 1970). Today it is therefore not so much to speak about the mucociliary transport in the respiratory tract. In cases of ineffective ciliary transport, secretion and particles may be found either intracellularly in the pharynx or extracellularly in the mucus. The mechanism of the cell or extracellular forces. Consequently, the mucociliary transport velocity and the wave movements ought to be used to study ciliary transport and ciliary beat frequency in connection with direct and indirect methods of ciliary activity in the respiratory tract.

In a previous paper (Mercke et al., 1970) a method is described for making objective recordings of the variations of light reflections from the mucous membrane about by cilia on an illuminated mucous membrane (Fig 4). In that paper it is also pointed out that the reflections reproduced from the movements within the mucous layer and the reflections may appear either on the surface of the mucous layer or on the tops of the cilia. Which theoretical and experimental backgrounds can be quoted for these two possibilities?

The mathematical deduction illustrated in Figs 1-3 shows the general relationship between recorded surface reflections and wave movements on a fluid surface. The recordings of surface reflections from regular surface wave movements in Newtonian fluids, e.g. water, are also in good agreement with the calculations (Fig 6). The ciliary

posed to be approximately valid for wave  
nents in mixed fluids like tracheo bronchial  
ons. However, it cannot be excluded that  
f the incoming light is refracted through  
n layer down to the carpet of moving  
In such a case a second type of wave  
must be taken into consideration.

aim of this investigation has been to  
re proposed two origins of surface light  
. The problem has been studied by  
rative experiments on ciliated mucous  
ranes and water surfaces. Wave movements  
frequency are induced on a water  
with the aid of a tone generator and a  
probe. The waves are propagated in rings  
the surface, and the light reflected from  
aves is recorded by the photomultiplier  
d (Fig. 6). This experiment shows the  
lity of the method for reproducing wave  
s accurately. These induced waves are  
duced accurately for amplitudes and fre-  
quencies. The wave reflections obtained from  
rabbit trachea, however, are irregular in these  
aspects. Frequency as well as amplitude  
variations are always seen in the biological  
material (Fig. 5A). Furthermore the irregular  
variations from the trachea may derive from at  
least two reflection points—the mucous surface  
and the underlying carpet of cilia. This is shown  
by the following experiments:

When a thin plastic film is placed on the  
mucous membrane in front of the microscope  
field the secretional waves are flattened  
and the reflection amplitudes decrease. At  
the same time the mean frequency increases  
(Fig. 5B). The light reflections are reduced but  
it is still possible to record the waves through the  
film. When the slightly heavier piece of glass  
is used as a covering on the membrane, the  
amplitudes are further reduced. The mean  
frequency is also reduced (Fig. 5D). However,  
after 15 min there is a frequency increase maybe  
biological response to a 'foreign body'  
on the film (Fig. 5E).

When the plastic film and piece of glass are  
in turn on a water surface, the ridges and  
troughs are equalized so that all reflection

variations disappear (Fig. 7). In the correspond-  
ing biological experiments, however, reflections  
are still recordable (Fig. 5). These results are  
therefore in accordance with our hypothesis that  
the light reflections emanate from the free ends  
of cilia as well as from the secretion layer.

Biorheological model experiments on ciliary  
mucous membranes have been made by Miller  
(1966) and by Barnett & Miller (1966). These  
authors are of the opinion that the ciliary wave  
movements can be classified as "trochoidal".  
This means that the troughs are longer than  
the ridges. They have also expressed the pre-  
sumption that this difference could be the  
basis of secretion—and particle transportation.  
The present authors have not, however, been  
able to verify this opinion. Instead we are  
inclined to believe that amplitude and frequency  
shifts, which appear on the records, are due to  
biorheological forces of the mucus and bio-  
chemical reactions within the ciliary cells. This  
problem of the combined influence of individual  
factors listed above will be the object for further  
investigations.

## ZUSAMMENFASSUNG

Mittels einer standardisierten Mikroskop-Photomultiplikator-Technik sind in *in vitro* Experimenten die

konnte nachgewiesen werden, dass sich die Oberflächen-  
reflexe von respiratorischer Schleimhaut aus mindestens  
zwei Komponenten zusammensetzen und zwar dem  
Reflex von der Schleimoberfläche und dem vom Flim-  
merhaarsteppich unter der Oberfläche.

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## THE INFLUENCE OF TEMPERATURE ON MUCOCILIARY ACTIVITY

*Temperature range 40°C–50°C*

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(Received February 13, 1974)

*act* *In vitro* experiments have been performed on us membranes from rabbit trachea in order to agate the relationship between the mucociliary wave frequency and an increase of temperature above body nature. The following results have been obtained. Optimum mucociliary activity was noted at or near normal body temperature. A further temperature rise 40° to 50° C did not noticeably influence the mucociliary wave frequency.

Mucociliary wave movements can be recorded during 0 minutes at 49° C. The critical temperature for irreversible ciliostasis was estimated to be about 51°–52° C. The "mucociliary survival time" of mucociliary activity *in vitro* experiments decreased from about 5 hours at 40° C to about 1 hour at 49° C.

In a previous paper (Mercke et al., 1974b) the mucociliary wave frequency in the rabbit trachea has been studied at temperatures between 20° and 40° C. An almost linear temperature/frequency relationship was found in contrast to other biological activities, which usually increase exponentially with increasing temperatures in this range (Guttman, 1969).

The mucociliary activity of the respiratory tract may be influenced and even destroyed at temperatures above 40° C. Febrile illnesses for example are often complicated by an increased mucociliary dysfunction and an insufficient elimination of secretions. It is therefore of theoretical as well as practical interest to study mucociliary function in an environment above the normal body tem-

perature. Only a few consistently performed experiments have hitherto been published regarding this problem.

As early as in 1866 Roth observed that cilia from the respiratory tract of rabbits stopped beating at a temperature of 45.5°–46.5° C. At 49° C the condition became irreversible. The transportation rate of the mucociliary system was studied in guinea pig trachea by Gordonoff & Mauderli in 1936. These authors found an upper limit of transportation at 42° C. The ciliary beat frequency was studied in *in vitro* experiments by Proetz (1934) and Iravani (1967) and their conclusions appear in Table I. Proetz and Tanaka (1967) have studied the temperature influence on mucociliary activity *in vivo*. However, their results differed a great deal and their methods were incomparable (Table I).

The duration of exposure must also be taken into consideration, for example in the case of applied research where mucociliary activity is chosen as an indicator of the effect of pharmacological and air pollution substances.

The aim of the present study has therefore been

1. to analyse the relationship between mucociliary wave frequency and increasing temperatures in the temperature range 40°–50° C and

2. to find the maximum duration of exposure for maintained activity at a few temperature levels between 40° and 50° C in *in vitro* experiments.

This investigation has been supported by grants from the Swedish Medical Research Council, Project No. B73 14X-01 and Project No. B74-61P-4282-01.

Table I

C = Cinematographical method    S = Stroboscopical method    P = Photoelectrical method

Authors	Method	Animal	<i>In vitro</i>	<i>In vivo</i>	Temperature for maximal frequency (°C)	Temperature for ciliostasis (°C)
Proetz, 1934	C	Rabbit		x	18-33	43-44
	C	Homo	x		18-33	43-44
Iravanu, 1967	S	Rat	x		38-40	42-44
Tanaka, 1967	P	Rabbit		x	ca 30	—

## METHOD AND MATERIALS

The standardized method for indirect recordings of the mucociliary activity via surface light reflections has been described in detail in a previous paper (Mercke et al., 1974a). Specimens of rabbit trachea mounted in a special experimental chamber (Mercke, 1974) have been used for *in vitro* experiments in an atmosphere above 90% relative humidity and temperatures varying between 40° and 50°C.

Two experimental series have been carried out

1 Tracheal specimens from 9 rabbits have been exposed to continuously increasing temperatures. The mucociliary wave frequency was recorded at 2° intervals between 40° and 50°C.

2 Tracheal specimens from 25 rabbits have been exposed to constant temperatures of 40°, 42°, 45°, 47° and 49°C. Five tracheae were used at each temperature level. Recording was continued until no further surface activity could be observed in the microscope. The duration ("mucociliary survival time") of retained activity was noted.

## RESULTS

### 1 Rabbit tracheae exposed to increasing temperature

The temperature of the specimen and of the surrounding air was continuously raised from 40° to 50°C during a mean time of 48 minutes (min 27 minutes, max 75 minutes). Fig. 1 illustrates typical recordings from one of the nine experiments at six different temperatures. The mucociliary wave frequency does not seem to change

very much when calculated directly from series of recordings. The results were also analysed by computer in order to find out the frequency range. Examples for one of the 40° and one of the 50°C levels are illustrated and revealing no increase in wave frequency with increasing temperatures. The frequency is about  $\pm 2$  Hz in both cases.

The wave frequency/temperature relationship was also plotted on diagrams which were processed by a computer. A binomial regression analysis for this relationship is shown finally at Fig. 3. The mucociliary wave frequency differs very little from 40°C (1060 waves/min) to 50°C (1130 waves/min). The amplitude pattern maintains roughly the periodical rhythm during the whole temperature range (Fig. 1).

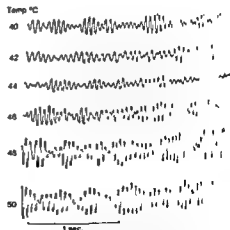
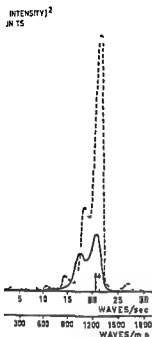


Fig. 1 Mucociliary wave frequencies recorded from a tracheal specimen at different temperatures between 40° and 50°C.



2 The relationship between mucociliary wave frequency at 40°C (continuous line) and 50°C (broken line) reflected light intensity in the sequence also shown in Fig. 1

#### *labbt tracheae exposed to constant temperatures*

heal specimens from 25 rabbits were exposed to constant temperatures of 40°, 42°, 45°, 47° and 49° respectively. At each level 5 rabbits were examined. The duration of mucociliary function is shown in Fig. 4. The duration of the activity increased with increasing temperatures. There was a statistically significant difference between 40° and the 49°C "mucociliary survival time" (Mann-Whitney rank-sum-test,  $p < 0.01$ ).

Wave frequency was sampled at 10 minute intervals for the 40°, 42° and 45°C levels and at 5 minute intervals for the 47° and 49°C levels. The results are seen in Table II.

A regression line for each temperature level was calculated by computer. The results appear in Fig. 5. The duration is only for the period in which at least 3 out of 5 specimens showed any activity at all. It will be noticed that the initial mucociliary wave frequency at every temperature is concentrated within a small range (1060–1200 waves/min).

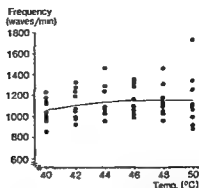


Fig. 3 Binomial regression line for all wave frequencies and temperatures in the range 40°–50°C from 9 animals

With increasing exposure time in constant temperature experiments, the mean frequency decreases very slowly at all levels except the 47°C level. It is also obvious that the slopes of the regression lines are very similar and nearly horizontal.

## DISCUSSION

The relationship between mucociliary activity and temperatures from 40° to 50°C has been investigated in order to determine (1) the temperature range for maximum activity, (2) the maximum temperature for maintenance of any activity at all, and (3) the "mucociliary survival time" for tracheal specimens at different temperature levels in *in vitro* experiments. The same method of investigation has previously been used

Temp.	Survival time						Mean value (minutes)
49°C	▲	▲	▲	▲			60
47°C		▲	▲	▲	▲		112
45°C			▲	▲	▲		133
42°C				■	■	■	258
40°C				■	■	■	307
	60	120	180	240	300	360	420 min

Fig. 4 The relationship between mucociliary survival time (the time during which activity can be recorded from the mucous membrane) and different temperatures in the range 40°–49°C.

Table II

40° C		42° C		45° C		47° C		49° C	
Mean sampling time (min)	Mean frequency (waves/min)	Mean sampling time (min)	Mean frequency (waves/min)	Mean sampling time (min)	Mean frequency (waves/min)	Mean sampling time (min)	Mean frequency (waves/min)	Mean sampling time (min)	Mean frequency (waves/min)
12	978	10	886	10	934	10	1 217	10	1 124
22	1 108	20	930	19	838	15	1 096	15	1 118
32	1 004	30	877	28	964	20	1 144	20	1 127
43	1 020	40	919	38	1 103	25	1 212	25	1 177
53	969	50	1 072	48	1 086	30	1 231	30	1 180
63	942	60	947	58	1 034	35	1 229	35	1 118
73	1 094	70	1 016	68	950	40	1 204	40	1 112
83	1 026	80	1 104	78	942	45	1 102	45	1 112
93	994	90	1 021	88	904	50	1 133	50	1 112
103	920	100	998	98	918	55	1 135	55	1 071
113	978	110	924			60	1 210		
123	1 041	120	1 004			65	1 187		
134	950	130	1 028			70	1 126		
144	1 080	140	1 175			75	1 233		
153	1 072	150	995			80	1 207		
163	1 025	160	813			85	1 298		
173	980	170	809			90	1 199		
183	956	180	825			95	1 170		
193	971	190	813			100	1 330		
203	929	200	675						
213	930	210	885						
223	948	220	846						
233	969	230	880						
243	882	240	796						
253	1 218								
263	1 049								
273	1 085								
283	959								
293	930								
303	851								
313	884								
323	837								
333	914								

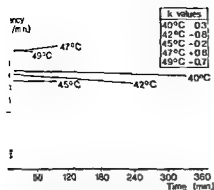
analogous experiments in the temperature range of 20° to 40° C (Mercke et al., 1974b)

The temperature borderline between the two investigations is just above the body temperature of rabbits, which is 37°–39°C according to Dalhamn (1960) and Altman & Dittmer (1966)

Between 20° and 30°C there was a mucociliary wave frequency increase of 300 waves/min which means a  $Q_{10}$  value of 2.1. In the next 10°C range the frequency increase was somewhat smaller i.e. a  $Q_{10}$  value of 1.4. The present results show no further frequency increase following a continuous temperature rise to 50°C. As seen in Fig. 3 there was no mucociliary frequency difference from 40°C (1060 waves/min) to 48°C (1140 waves/min). The  $Q_{10}$  value of this range was 1.1.

This means that the mucociliary wave movement have already reached their maximum at body temperature and that a further temperature increase from 40° to 50°C has hardly additional influence on this activity. No temperature peak for maximum activity can be seen. Even the mucociliary wave pattern remained unchanged during the temperature increase (Fig. 1). However, the validity of conclusions must be confirmed in *in vivo* experiments.

Below body temperature the wave frequency is influenced by two factors. One is the cellular "pacemaker" rhythm. Another is the rheological property of the mucus (Tomotake et al., 1974). Both these parameters are



1. Regression lines for five different temperatures in range 40°–49°C. The lines represent that part of the experiment in which at least 3 out of 5 tracheal specimens still alive.

as a result of temperature changes. The logical factor—which is said to decrease with increasing temperatures (Adamson, 1967)—is very important at low temperatures obviously has less influence above 40°C even the intracellular activity seems to be related above body temperature but this existing observation ought to be tested with recordings of the intracellular electrical activity (Adamson & Toremalin, 1966) at varying temperatures.

In the series of constant temperature experiments there is an initial increase in wave frequency at 42° and 45°C.

In the other series, however, the wave frequency is continuously high until the activity suddenly disappears (Table II). The reduced "mucociliary survival time" of the tracheal specimens *in vitro* at high temperatures may be due to changes of the biochemical processes. What is the temperature level for irreversible mucociliary activity? Proetz (1934) found such maximum temperature at 43°–44°C in rabbits during *in vivo* experiments and the same temperature range *in vitro* in human tissues. However, humidity factor was not sufficiently considered in these experiments. Iravani (1967) is of the opinion that 42°–44°C should be looked on as the critical temperature. If an extrapolation is made from the present results (Fig. 1) the critical temperature appears to be at 51°–52°C.

A regression line has been calculated by a computer (Fig. 5) for each temperature on the basis of the mean frequencies for each measuring interval. The regression lines are practically parallel and almost horizontal. This means that the initial mucociliary wave frequency is maintained over the entire range from 40° to 49°C without any distinct frequency peak.

The present results are of little interest from a clinical point of view. However, the constant temperature experiments can be valuable for applied experimental purposes because the maximum duration of such experiments should not exceed 5 hours at 40°C, 4 hours at 42°C, 2 hours at 45°C and 47°C and 1 hour at 49°C.

## ACKNOWLEDGEMENT

I am greatly indebted to Daniel Huberman, B.Sc., for valuable laboratory assistance.

## ZUSAMMENFASSUNG

Bei *in vitro* Versuchen an der Trachealschleimhaut von Kaninchen ist die Abhängigkeit der mukoziliären Wellenfrequenz von Temperatursteigerungen über Körpertemperaturniveau untersucht worden. Die Versuche ergaben folgende Resultate:

1. Optimale mukoziliäre Aktivität erfolgte bei oder nahe bei normaler Körpertemperatur. Eine Erhöhung der Temperatur von 40°C auf 50°C hatte keinen feststellbaren Einfluss auf die mukoziliäre Wellenfrequenz.

2. Mukoziliäre Wellenbewegungen konnten bei 49°C während 60 Minuten registriert werden. Die kritische Temperatur für irreversibles Erlöschen der Flimmeraktivität wird auf ungefähr 51°C–52°C geschätzt.

3. Die mukoziliäre Überlebenszeit bei *in vitro* Versuchen sank von ungefähr 5 Stunden bei 40°C auf zirka eine Stunde bei 49°C.

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## PLETHYSMOGRAPHIC STUDIES OF THE BLOOD FLOW IN THE MUCOSA OF THE HUMAN MAXILLARY SINUS

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(Received February 4, 1974)

Plethysmography of the mucosa of the maxillary has been possible due to following facts and obser-

The maxillary sinus is an air filled cavity with com-

ly rigid walls

Its ostium can be completely blocked experiment

The surface area of the mucosa in the maxillary can be calculated roentgenographically

Pulse waves are sometimes seen during pressure re-

gins from maxillary sinuses with obstructed ostium

Similar pulse waves are almost always present in

sal sinuses with experimentally closed ostium.

An upward slope of the pulse wave recording is

used during compression of the jugular veins

All errors involved in such plethysmographic re-

ings seem to result in an underestimation rather than

overestimation of the blood flow The mean blood

obtained by measurements during bilateral jugular

pression was  $0.26 \mu\text{l}/\text{cm}^2 \text{ sec}$  With an estimated

total thickness of  $125 \mu\text{m}$  the blood flow will be  $125$

$00 \text{ cm}^3 \text{ min}$ . This would mean that the blood flow

of maxillary mucosa is greater than in muscle brain

liver and only surpassed by the kidney and lungs

duction of mucosal thickness and of the pulse waves

obtained during compression of the carotid artery

blood flow of the mucous membranes in

upper respiratory tract is influenced by differ-

stimuli of an emotional, thermal, or physical

re It has also been proposed that there

is an abnormal mode of blood flow and

of vessel reaction in some nasal diseases,

especially vasomotor rhinitis (Godm, 1961,

Wistedt & Runderantz, 1964) Animal experi-

ments have shown that the blood flow of the

nasal mucosa changes in different ways in re-

sponse to different stimuli (Naumann, 1961)

Animal experiments with qualitative studies of the blood flow have contributed considerably to our present knowledge of the blood flow of the respiratory mucosa

To obtain a more exact understanding of the blood flow of the mucosa in man it is necessary to have quantitative methods for studying the blood flow Photoelectric plethysmographic studies of the nasal mucosa (Davis & Hertzman, 1957, Kato, 1965, Yamakawa, 1967) have given valuable information on the blood flow but so far only qualitative studies have been published

Thermal conductivity measurements of the nasal blood flow have been performed as semi-quantitative measurements (Drettner, 1961, Schroeteler, 1964) However, no quantitative measurements of the human mucosal blood flow in the upper respiratory tract, nor in any other human mucosa in vivo, have been published This lack stands in contrast to the multitude of reports of quantitative blood flow studies in the skin, muscle, etc The lack of mucosal blood flow studies is obviously a consequence of the difficulties encountered in devising methods for such investigations

The purpose of this work is to present preliminary results of a new method for the quantitative study of blood flow of the maxillary sinus mucosa

The blood flow of the mucosa of the paranasal sinuses deserves to be studied since the mucosal swelling—well known in several diseases of an infectious, allergic, or vasomotor nature—must to some extent be connected with blood flow

Work was supported by the Swedish Medical Research Council (project 749)



changes. Furthermore all calculations of the gas exchange in the paranasal sinuses will be based on a more solid ground if they are combined with blood flow measurements in the mucosa.

### THEORETICAL ASPECTS

The maxillary sinus, and to some extent also the frontal sinus, has a unique property for plethysmographic blood flow studies in comparison with other organs containing a mucosa, namely a cavity with completely rigid walls except for the ostium which is small and can be obstructed experimentally or spontaneously. Furthermore, it has a mucosa of which the surface area can be calculated. In order to study the patency of the maxillary ostium the pressure within the maxillary sinus is often recorded and during such recordings pulse waves are sometimes seen in the recordings. In almost all healthy sinuses with experimentally obstructed ostium this pulse wave was seen. Such pulse waves were described by Drettner (1965) and they were observed occasionally in patients with total or partially obstructed ostia, though not in those with patent ostia.

Recording of these pulse waves can be used for plethysmographic measurements if the blood flow from the sinus can be essentially stopped for a few seconds and if the volume of the sinus can be measured. A method for the latter has been elaborated (Aust & Helmius 1974). Furthermore, it is necessary to have information about the ostial patency, which is easily available (Drettner, 1965).

The blood vessel to the maxillary sinus belongs, on the arterial side, principally to the external carotid system and on the venous side to the internal and external jugular systems, while the vertebral arterial and venous systems do not supply the mucosa (Wagemann, 1964). In that way blockage of the arterial or venous supply to the mucosa will be possible by compression on the neck but some blood flow to or from the vertebral system or other non blocked systems, cannot be completely avoided. The short interval—only a few seconds—during which the

blockage is performed will probably not affect the arterial supply or venous outflow to the sinus more than the ordinary ways.

### MATERIAL AND METHODS

Ten persons (age range 20–63 years) were investigated. All of these subjects were healthy with normal rhinoscopy and normal roentgenograms of the paranasal sinuses.

The investigation was performed on each subject in a semirecumbent position. The pressure within the maxillary sinus was recorded by introduction of a cannula with a side hole into the sinus. The instruments used were a pressure transducer (EMT 33, Elema-Schönander, Stockholm), an amplifier (EMT 31) and a recorder (Mingograph 34). The obstruction of the ostium was caused by swollen mucosa or by a tamponade checked by lowering and raising the pressure (about 5 cmH<sub>2</sub>O) in the sinus and measuring the volume with a syringe connected to the manometer. The volume of the sinus was measured by the roentgenographic method (Aust & Helmius 1974).

Compression of the carotid artery was performed manually, using two fingers and a strong pressure. The internal and external carotid veins were compressed together as somewhat more lateral, yet not so forceful, compression. A few trials were usually necessary before a selective and effective compression was obtained. All compressions were performed rapidly and lasted only a few seconds. Three measurements were performed and the mean of the used.

In order to calculate the blood flow from the surface area it was necessary to calculate the surface of the mucosa of the maxillary sinus. This was done by using the pressure obtained during roentgenographic measurement of the sinus volume. A mucosa which is (or only a slight) swelling seems to be a prerequisite for the calculation of the surface area (Aust & Helmius 1974).

Information on the patency of the maxillary ostium was obtained by simultaneous

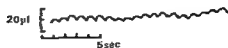
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Age	Ostial patency	Sinus volume Roentgen ologically (ml)	Mucosal surface (cm <sup>2</sup> )
20	Patent	10.26	36.60
28	Patent	22.22	63.54
25	Patent	17.69	48.23
26	Patent	15.23	45.11
27	Patent	13.04	41.17
20	Patent	16.88	48.70
63	Patent	13.40	40.70
20	Patent	16.56	47.94
27	Patent	12.29	41.41
34	Patent after blowing	14.93	43.95
		15.20	45.79

findings in the maxillary sinus and nasal cavity during breathing, blowing and sniffing (Drett-1965)

## RESULTS

Fig. 1 shows that the maxillary ostium was patent in 9 of the subjects and became patent after blowing of the nose in the last subject. The volume of the maxillary sinus measured roentgenographically is given from Table I. The surface area of the mucosa in the maxillary sinus is also presented in that table. An example of a pulse wave recorded from the maxillary sinus is shown in Fig. 1. The magnitude of the pulse waves in all 10 subjects correlated to a mean volume increase of  $8.2 \mu\text{l}/\text{beat}$  or  $17 \mu\text{l}/\text{sec}$ . This represents the blood added



5  $\mu\text{l}$  blood/heart beat/total antral mucosa  
0.1  $\mu\text{l}$  / /cm<sup>2</sup>

1  $\mu$  increase of mucosal thickness/heart beat

Fig. 1 Pressure variations in the maxillary sinus due to pulse waves in the vessels of the antral mucosa in a healthy maxillary sinus with an experimentally closed ostium. Calculations of the increase in blood flow due to these pulse waves.

to the vessels by the systolic action of the heart while the basal blood flow is not taken into account.

When the jugular veins were compressed on both sides the slope of the plethysmographic recording could be used for blood flow measurements (Fig. 2). The mean increase measured for the total antral mucosa was  $11.6 \mu\text{l}/\text{sec}$  during bilateral jugular compression ( $n=7$ ) and  $7.5 \mu\text{l}/\text{sec}$  during ipsilateral jugular compression ( $n=7$ ), while contralateral jugular compression gave a mean increase of  $1.8 \mu\text{l}/\text{sec}$  ( $n=7$ ). When the value measured during bilateral jugular compression is correlated with the mucosal surface area, a mean of  $0.26 \mu\text{l}/\text{cm}^2 \text{ sec}$  is obtained. The thickness of the mucosa of the sinuses is reported in the literature (Loring & Tenney, 1973) to be  $125 \mu\text{m}$ . A blood flow of  $20.9 \mu\text{l}/\text{cm}^3 \text{ sec}$  is thus obtained for the mucosa or  $125 \text{ ml}/100 \text{ cm}^3 \text{ min}$ .

The maximal increase of the antral mucosa during a Valsalva manoeuvre was also measured

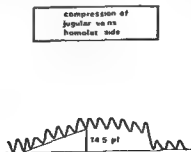
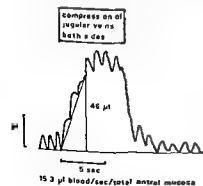


Fig. 2 Pressure rise in the maxillary sinus at bi- and unilateral compression of the jugular veins.



Fig 3 Pressure rise with intact pulse waves in the maxillary sinus during Valsalva manoeuvre

(Fig 3) The mean increase was  $25.2 \mu\text{l}$  for the antral mucosa in 5 subjects, or  $0.58 \mu\text{l}/\text{cm}^2$ . When using a value of  $125 \mu\text{m}$  for the thickness of the antral mucosa an increase of  $46.4 \mu\text{l}/\text{cm}^2$  was obtained.

Plethysmographic recording during compression of the common carotid artery of the same side was performed on 2 subjects (Fig 4). A vanishing of the pulse wave and a downward slope of the plethysmographic recording was obtained. The mean decrease in volume of the total antral mucosa was  $14.1 \mu\text{l}$ , or  $0.26 \mu\text{l}/\text{cm}^2$ .

## DISCUSSION

This appears to be first quantitative measurement of the blood flow of any mucosa in man. However, the magnitude of the error due to incomplete interruption of all blood flow from the sinus during the jugular compression cannot be evaluated. An outflow through the vertebral or other non-blocked veins cannot be completely excluded but was probably small when the jugular compression lasted only a few seconds. It is also impossible to be sure that all blood flow in the jugular veins was blocked during the compression. These errors work in the same direction, implying that the values of blood flow obtained during bilateral jugular compression are probably too low. There can hardly be any



Fig 4 Pulse wave recording from the maxillary sinus during compression of the carotid artery on the homolateral side. Pronounced reduction of the waves and a decreased pressure level in the sinus indicating reduction of the thickness of the mucosa.

Table II. Results of antral plethysmography during jugular compression

Case no	Slope during jugular compression			Blood flow ( $\mu\text{l}/\text{cm}^2/\text{s}$ )
	Contralateral compression ( $\mu\text{l}/\text{sec}^{-1}$ )	Homolateral compression ( $\mu\text{l}/\text{sec}^{-1}$ )	Bilateral compression ( $\mu\text{l}/\text{sec}^{-1}$ )	
1	0	1 735	—	—
2	0	4 050	4 725	0.074
3	0	11 330	11 540	0.241
4	—	—	—	—
5	3 17	5 433	5 890	0.141
6	2 15	4 300	10 010	0.209
7	4 63	3 700	20 340	0.500
8	—	—	—	—
9	2 61	13 020	13 560	0.175
10	—	12 930	14 980	0.254
Mean	1 79	7 460	11 570	0.24

error of importance in the opposite direction caused by these factors. The compression of the air in the sinus, tubes of the recording system and pressure transducer is a factor which may cause an error in the recordings. However, as in all plethysmographic studies with a closed system this factor is already considered in the calibration (Graf & Westersten 1957). An error of importance only occurs during rapid pressure changes. Furthermore, this will therefore only give an underestimation or an overestimation, of the blood flow.

A back flow of the blood might theoretically be possible since the veins have no valves. However, the only blood which may flow backward is that compressed by the fingers during the jugular compression.

Table III. The blood flow in some human tissues according to Textbook of Physiology and Chemistry (1959) and Handbook of Reference Values (1958). The value of the mucosa of the maxillary sinus is obtained from the present investigation.

Tissue	Blood flow ( $\text{ml}/100 \text{ g}^{-1} \text{ tissue}/\text{min}$ )
Muscle, forearm, rest	2
Muscle, forearm, exercise	30
Brain	65
Liver	100
Mucosa, maxillary sinus	125
Kidney	400
Lungs	450

overflow from other organs in the head occur but it must be small during a measured period of 3 seconds especially since the vessels have a parallelly and not a serially connected circulation. In roentgenographic investigations of the blood flow in the neurocranium there is never any overflow to the vessels of the extracranial organs even when the jugular veins are compressed (Bergstrom 1974). It may be of interest to compare the results of blood flow obtained in the mucosa of the maxillary sinus with values of blood flow in other organs. Table III shows reports from the literature concerning the blood flow in different organs. Using the approximation that a 100 cm<sup>3</sup> of antral mucosa has a weight of 100 g the blood flow of the antral mucosa is 60 times that of resting muscle, 4 times that of active muscle, 1/3 that of the brain, slightly more than that of the liver, while the kidney and the lungs have a higher blood flow than the antral mucosa. The antral mucosa therefore must be considered to have a large blood flow, which contradicts the assumption of Loring & Tenney (1973). It seems likely that the present method can be applied in experimental blood flow studies, for example in pharmacological or similar experiments.

## ZUSAMMENFASSUNG

Die Messung der Blutflussrate der Kieferhöhle ist bei folgenden Tatsachen und Beobachtungen möglich: Die Kieferhöhle ist ein luftgefüllter Hohlraum mit glatten Wänden. Ihr Ostium kann experimentell vollständig blockiert werden. Die Oberfläche der Kieferhöhlenschleimhaut kann roentgenographisch berechnet werden. Pulswellen sind manchmal während Druckregistrierung von der Kieferhöhle bei verschlossenem Ostium messbar. Ähnliche Pulswellen sind fast immer in normalen Nasenhöhlen mit tamponiertem Ostium vorhanden. Ein Ansteigen der Pulswellenregistrierung wird während Kompression der Jugularvenen erreicht. Alle Fehler, mit denen solche plethysmographischen Messungen behaftet sind, scheinen eher eine Unter- als eine Überschätzung des Blutflusses zu ergeben. Der durchschnittliche Blutfluss bei Messungen während der Kompression auf die verschlossenen Jugularvenen erreichte wurde war 0.26 ml/min. Bei einer geschätzten Schleimhautdicke von

125 µm ergibt sich ein Blutfluss von 125 ml/100 cm<sup>3</sup> min. Das würde bedeuten, dass der Blutfluss in der Kieferhöhlenschleimhaut grösser ist als im Muskel, im Gehirn und in der Leber und nur von dem in der Niere und der Lunge übertroffen wird. Eine Reduktion der Schleimhautdicke und der Pulswellen wird bei einer Kompression auf die A. Carotis communis erreicht.

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## OXYGEN TENSION IN THE HUMAN MAXILLARY SINUS UNDER NORMAL AND PATHOLOGICAL CONDITIONS

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(Received February 7, 1974)

**Abstract** The  $pO_2$  in the maxillary sinus measured with a small  $pO_2$  electrode was found primarily to be dependent on the patency of the maxillary ostium. It was also correlated with the functional size of the maxillary ostium when this was smaller than 2.5 mm in diameter. A mean oxygen tension of 116 mmHg was found in the maxillary sinus of normal cases and a mean antral  $pO_2$  of 75 mmHg in patients with sinusitis. The  $pO_2$  could not be shown to be related to the presence or absence of antral pus or mucus in the cases with sinusitis. However, oxygen tensions of only a few mmHg were found in two cases with aerobic bacteria in the antral pus. Pain in the region of the paranasal sinuses was to some extent correlated to low antral  $pO_2$ .

Change in the oxygen content in the paranasal sinuses after obstruction of the ostium has been associated with the pathophysiology of sinusitis and vacuum sinus (Sluder, 1927, Flottes et al., 1960).

A few experiments concerning oxygen changes in paranasal sinuses after ostial obstruction have been reported in the literature. A decreasing oxygen (and increasing carbon dioxide) tension in the frontal sinus of dogs was found by Doiteau (1955) after occlusion of the naso frontal duct. Studies in man with Scholander's method of aspirated air from the maxillary sinus showed a slightly lower oxygen tension in patients with sinusitis than in normal subjects, 16.60% and 17.29%, respectively (Kitayama, 1968). Aust & Drettner (1971) introduced a small  $pO_2$  electrode into the sinus in man and found, in a preliminary report, that the antral oxygen tension was correlated to the patency of the maxillary ostium.

This work was supported by the Swedish Medical Research Council (project 749).

The purpose of the present investigation was to analyse the oxygen tension in the maxillary sinus in relation to the clinical data, considering in particular the patency of the ostium, the functional size of the ostium, the volume of the sinus, the presence or absence of mucus in the sinus, recurrent sinusitis and occurrence of pain in the forehead or the

### MATERIAL AND METHOD

The series consists of 97 persons on whom investigations were performed (Table I). All subjects were investigated twice.

The oxygen tension in the sinus was measured with a small  $pO_2$  electrode (Brotzu & 1967, Aust & Drettner, 1972) introduced into the maxillary sinus through the inferior meatus after anesthesia with Xylocain (Astra). Calibration was performed according to a method previously reported (Aust & Drettner, 1972), with the exception that a temperature of 36.0°C was used instead of 37.0°C. In temperature measurements with thermocouples the human maxillary sinus showed a temperature of 36.0°C.

The patency of the maxillary ostium was investigated with simultaneous pressure measurements in the sinus and the nasal cavity by breathing blowing and sniffing (Drettner, 1971). The volume of the sinus was measured radiologically (Aust & Helander, 1971). The functional size of the maxillary ostium

# c I Presentation of the material

no.s	Number of subjects	Number of investigations
normal subjects	32	38
tracheostomies	3	3
ostio septi nasi	9	9
motor rhinitis	10	11
polyps	7	8
sinusitis	15	16
sinusitis (total)	5	6
recurrent acute sinusitis (active period)	5	5
recurrent acute sinusitis (silent period)	6	6
%	5	5

determined by pressure recordings in the maxillary sinus during momentary air insufflation with a known airflow (Aust & Drettner, 1974)

## RESULTS

Table II shows the oxygen tension in the different nosologic categories as seen in the table the average oxygen tension in the maxillary sinus of normal subjects was 14 mmHg or 16.3%. The group with nasal

Table II  $pO_2$  in the maxillary sinus in relation to nosology

no.s	Number of investigations	Mean $pO_2$		S.D. mmHg
		mmHg	%	
normal subjects	38	116.4	16.3	23.9
normal with patent ostium	32	117.6	16.5	32.6
tracheostomies	3	90.3	12.7	9.3
ostio septi nasi	9	105.6	14.7	40.8
motor rhinitis	11	106.5	14.9	41.4
polyps	8	79.5	11.2	28.5
sinusitis	16	74.8	10.5	48.7
sinusitis (total)	6	74.9	10.5	30.1
recurrent acute sinusitis (active period)	5	101.6	14.2	65.4
recurrent acute sinusitis (silent period)	6	99.0	13.9	40.3
%	5	140.5	19.7	23.4

disorders such as deviated septum and vasomotor rhinitis had a slightly lower  $pO_2$ .

The patients with acute sinusitis had a mean value of about 75 mmHg, with no distinction between dental and non dental sinusitis. The group with recurrent sinusitis had a mean oxygen tension of about 100 mmHg (14.0%) with no difference when measured in the silent or active period. A silent period is defined as an interval of at least 3 months during which the patient with recurrent sinusitis has been free from any acute exacerbations.

## Nasal breathing

In 3 patients without any nasal breathing but normal anatomy, in 2 patients who had undergone laryngectomy and one patient with a tracheostomy, the mean  $pO_2$  in the maxillary sinus was found to be 90.3 mmHg (12.7%).

## Ostial patency

The antral oxygen tension in relation to the patency of the maxillary ostium is shown in Table III.

The mean value with a patent ostium was 116.5 mmHg (16.3%) and for a partially patent ostium, 100.0 mmHg (13.6%). The cases with ostia functioning as valves had a mean value of 99.8 mmHg (14.0%) and those in which the ostia were obstructed even during blowing/sniffing had a mean value of 80.7 mmHg (11.3%). Cases in which patency was achieved after blowing and sniffing were too few for any conclusive results. It is interesting to note that there were no antral  $pO_2$  values below 30 mmHg in the groups with patent, partially patent, patent after blowing/sniffing or valvular ostia, while 5 patients with obstructed ostia had oxygen tensions below 30 mmHg and 4 of these were below 10 mmHg.

## Functional ostial size

The functional size of the maxillary ostium was measured in 24 normal sinuses in which  $pO_2$  was determined simultaneously. The mean  $pO_2$  of this group was 128.5 mmHg (18.0%). There was a positive correlation between the oxygen tension in the maxillary sinus and the size of the

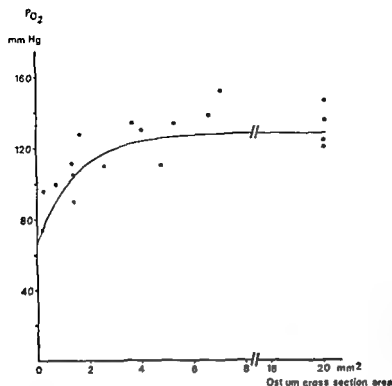


Fig. 1 Relationship between the maxillary sinus and the function of the maxillary ostium. The graph shows the relationship is a regression curve with a correlation coefficient of 0.88 measured data.

ostium up to a cross sectional area of about 5 mm<sup>2</sup> or a diameter of about 2.5 mm (Fig. 1). The line depicting the relationship between ostial size and antral pO<sub>2</sub> in Fig. 1 was mathematically calculated and has a correlation coefficient of 0.88. In addition it shows that the antral pO<sub>2</sub> is constant in sinuses with ostial cross sectional areas larger than 5 mm<sup>2</sup>.

#### Antral volume

The volume of the maxillary sinus calculated from roentgenograms (Aust & Helmius, 1974) was measured in 27 healthy persons in whom antral pO<sub>2</sub> also was determined. In addition in 18 of these persons the functional size of the maxillary ostium was measured manometrically. Analyses of the data from the examined group revealed no correlation between the pO<sub>2</sub> in the sinus and the antral volume.

#### Cases of Sinusitis

##### Presence or absence of mucus in the sinus

In 14 of the 16 patients with non dental sinusitis, antral washing was performed with saline accord-

ing to ordinary therapeutic methods. Some patients had purulent contents in the sinus. Of 7 patients had no antral pus. The mean pO<sub>2</sub> in sinuses with purulent contents was 75 mmHg (10.5%) while patients without antral pus had a mean pO<sub>2</sub> of 61 mmHg (8.6%). The difference was not statistically significant. Cases with purulent content had a higher metric pressure indicated by spontaneous flow of pus through the cannula for washing. In a few cases the oxygen ten-

Table III pO<sub>2</sub> in the maxillary sinus in relation to the patency of the maxillary ostium

Ostial status	Number of investigations	Mean pO <sub>2</sub>	
		mmHg	%
Patent	46	116.5	14.1
Partially patent	5	100.0	13.4
Patent after blowing or sniffing	3	100.7	13.3
Valve acting	13	99.8	13.0
Obstructed	27	80.7	11.3
Not investigated	13	93.8	12.2

IV Relationship between ostial status and  $pO_2$  in cases with recurrent sinusitis in and silent period

period	Silent period			
	$pO_2$		$pO_2$	
	mmHg	%	Ostial status	mmHg %
	92	12.9	Patent	154 21.6
	90	12.7	Patent	145 20.3
II	160		Partially patent	88 9.7
ded	163		Partially patent	
			65	9.1
ded	3	0.4	Valve acting	70 9.8
			Occluded	91 12.8

times very high, as seen in 2 cases in Table for example. The oxygen percentage in these was not known as the manometric pressure not measured.

etiology

In patients with inflammation and pus or as in the maxillary sinus had positive bacterial cultures taken from the antral washings the antral washing was classified with (-) to discharge and (+), (+ +), (+ + +) for according to amount, colour and viscosity. Table V shows the diagnosis, ostial status, bacterial and  $pO_2$  in the investigated sinuses. In this it is obvious that most maxillary sinuses

with an abundant purulent content usually had a lower  $pO_2$  than a sinus with less. However, one patient with (+ + +) in antral washing and patent ostium had a high antral oxygen tension.

In the table we can also see that there is a tendency to higher  $pO_2$  in the sinuses with more patent ostia than in those with occluded ostia.

Two patients with very low  $pO_2$  values, 5 and 6 mmHg, and (+ + +) in antral washings had purulent infections with aerobic bacteria. Two others had (+ + +) in discharge but antral  $pO_2$  of 65 and 84 mmHg. These patients had both dental sinusitis with anaerobic bacteria. However, only one of them had a completely occluded maxillary ostium.

Pain

In the series of patients with sinusitis, 9 patients had pain in the forehead or over the maxillary sinus. The severity of the pain was not investigated, nor was the manometric pressure in the sinus measured.

Pain in the forehead and sinuses can occur at greatly differing antral  $pO_2$  levels, but it seems to be most prevalent in cases with a low antral  $pO_2$  values (Table VI).

## DISCUSSION

This investigation is the first in which the oxygen tension in the maxillary sinus has been analysed.

Table V Findings in 11 patients with posttubercular bacterial cultures

nos	Ostial status	$pO_2$		Antral washing	Bacteria
		mmHg	%		
sinus acuta	Occluded	32	4.5		<i>Staphylococcus aureus</i>
sinus acuta	Occluded	62	8.7	-	<i>Staphylococcus aureus</i>
sinus acuta	Valve acting	92	12.9	+	<i>Staphylococcus aureus</i>
sinus recidivans fe exacerbation	Patent	147	20.6	- -	<i>Staphylococcus aureus</i>
sinus acuta	Occluded	6	0.8	+	<i>Haemophilus influenzae</i>
sinus acuta	Partially occluded	128	17.6	+	<i>Haemophilus influenzae</i>
sinus acuta	Occluded	5	0.7	-	<i>Flavobacterium</i>
sinus acuta	Occluded	134	18.8	+	<i>Staphylococcus albus</i>
sinus acuta dental	Occluded	51.5	7.2	-	<i>Pyococcus</i>
sinus acuta dental	Partially occluded	65	9.1	+++	Gram pos anaerobic coccus
sinus acuta dental	Occluded	84	11.8	+++	Gram pos anaerobic coccus



Table VI  $pO_2$  in relation to pain in 9 patients with sinusitis

Ostial status	Discharge	$pO_2$	
		mmHg	%
Occluded	-	3	0.4
Occluded dental fistula	+++	52	7.3
Valve acting	+	57	8.0
Occluded	+	69	9.8
Occluded	-	75	10.5
Patent	-	75	10.5
Occluded	-	80	11.2
Occluded	+	128	18.0

in a large series of persons with a method avoiding most of the problems of previously used techniques. Some technical problems have been encountered, however. Whilst introducing the cannula with the small  $pO_2$  electrode through the bony wall of the lower nasal meatus into the sinus, the electrode membrane sometimes varied its position, thus altering the calibration. A new calibration was therefore performed after each experiment and if the values differed by more than 20 mmHg, the experiment was abandoned. A difference of 20 mmHg was accepted since a small drift could occur during the hour's time usually required (Aust & Drettner, 1972) for these measurements.

The mean antral oxygen tension in all normal subjects was 116.4 mmHg (16.3%) while in those where it also had been shown that the maxillary ostium was patent, 117.6 mmHg (16.5%).

Flottes et al. (1960) reported an oxygen content of 17.5% in the frontal sinus of normal dogs, which is almost identical with our measurements in living man.

Two factors can be thought to cause a decrease in the oxygen tension of the maxillary sinuses, namely the low  $pO_2$  of the expiratory air and the absorption of oxygen in the antral mucosa. The absorption in the mucosa can be counteracted by adequate ventilation through the ostium requiring a patent ostium. This ventilation is also influenced by the nasal respiration (Aust & Drettner, 1971).

Patients without nasal breathing, but with

normal nasal anatomy and patent ostia, in our investigation 3 persons who had a tracheostomy or laryngectomy) had a  $pO_2$  which was lower than in normal subjects, probably due to the lack of nasal respiration, consequently a reduced ventilation of the ostium. The number of patients with nasal respiration was, however, too small for conclusions.

The investigation showed that there is a positive correlation between the antral  $pO_2$  and the functional size of the ostium up to a sectional area of 5 mm<sup>2</sup> (ostium diameter 2 mm). In maxillary sinuses with still smaller ostia the  $pO_2$  was found to be about 10% regardless of ostial size which implies the sinus is sufficiently ventilated to counteract oxygen absorption in the sinus. In smaller ostia, the  $pO_2$  is lower and approaches to the functional size of the ostium the  $pO_2$  approaches to zero. At the ostium the  $pO_2$  is 70 mmHg (Fig. 1). This value is far from the mean value found in patent maxillary sinuses, 80.7 mmHg.

A relative insufficiency of antral ventilation is thus present at ostial diameters less than 2 mm.

It seems likely that factors which are of relative small importance for antral ventilation, e.g. decrease in nasal breathing, the actual side of the nose, for example, deviations or variations caused by the nasal cycle, can be of importance for the antral ventilation when the ostium is small.

Patients with sinusitis had a low  $pO_2$  compared with normal subjects. The difference in patients with non-dental infections. Neither was there any difference in cases with or without pain in the sinus. The  $pO_2$  in the sinus was partially related to the patency of the ostium.

Patients with recurrent acute sinusitis both in active and in silent periods had a low  $pO_2$  which was principally correlated to the patency of the ostium. In both the periods of acute exacerbations of the recurrent sinusitis and in those in the silent period, we found both patent and occluded ostia.

representation in both groups of non-patent. A correlation between disturbed patency of the ostium and recurrent sinusitis seems probable.

The relationship between the bacteriology in the sinus and the  $pO_2$  of the sinus was studied in patients with acute sinusitis. In a few cases of aerobic bacteria, the antral  $pO_2$  was close to zero while no cases with anaerobic bacteria had such low values. However, a very low antral  $pO_2$  (3 mmHg) was also found in a case without any bacteria in the sinus nor any bacteria in the antral washing.

The low  $pO_2$  in sinuses with small ostia or disturbed ostial function can perhaps be a factor in the development of sinusitis. It has been shown in animal experiments that if nitro-oxygen was substituted for air in occluded tracheal catheters, the transportation of mucus by the cilia in the tracheal mucosa was decreased (Flottot et al., 1960). The effect was reversible and when oxygen was introduced again the mucus transportation improved. Possibly a low  $pO_2$  in a paranasal sinus can have the same effect on the cilia of the nasal mucosa and be a factor in the development of sinusitis.

Low vacuum sinus has been discussed in connection with a low manometric pressure in the sinus (Sluder, 1927; Söderberg, 1934; Ballenger & Ballenger, 1952) and with low oxygen and low carbon dioxide content in the sinus (Flottot et al., 1960). The low pressure is thought to be a pain factor. In our investigation there were 7 patients with sinusitis combined with pain in the paranasal region. Six of these had reduced partial oxygen tension in the sinus and only one of the patients had a purulent discharge on antral washing. A correlation between low oxygen content and pain in the sinus may thus be possible but it is not the only explanation. The manometric pressure could not be measured in these patients for technical reasons.

der Öffnung des maxillaren Ostiums und auch von der funktionellen Grösse des maxillaren Ostiums abhängig, wenn dieses einen kleineren Durchmesser als 2.5 mm hat. In normalen Fällen wurden in der Kieferhöhle eine durchschnittliche Sauerstoffspannung von 116 mmHg und bei Patienten mit Sinusitis ein durchschnittlicher  $pO_2$  von 75 mmHg festgestellt. Es wurde keine Korrelation zwischen dem Volumen und dem  $pO_2$  der Kieferhöhle festgestellt. Der  $pO_2$  bezog sich in Fällen mit Sinusitis nicht auf das Vorkommen und Nichtvorkommen von Eiter oder Schleim in der Kieferhöhle. In zwei Fällen mit aeroben Bakterien im Eiter der Kieferhöhle wurde jedoch eine Sauerstoffspannung von nur wenigen mmHg gemessen. Bei Schmerzen in der Gegend der Nebenhöhlen wurde manchmal ein niedriger  $pO_2$  festgestellt.

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## ZUSAMMENFASSUNG

$pO_2$  in der Kieferhöhle gemessen mit einer  $pO_2$ -Elektrode ist, wie grundlegend festgestellt wurde, von

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COMBINED RADIOLOGICAL AND SURGICAL THERAPY  
OF CANCER OF THE ETHMOID

Å Elner and Hj Koch

*From the ENT Clinic, University Hospital Lund Sweden*

(Received February 12, 1974)

**Abstract** A material of 12 cases of cancer of the ethmoid is presented. The treatment was combined radiology and surgery with pre- and eventually postoperative radiotherapy and lateral rhinotomy with resection of the anterior base of the skull and dura mater in cooperation with a neurosurgeon. Craniotomy of the conventional type was not performed. In spite of the limited number of cases the results are considered promising and encourage treatment along the lines described in the article. 9 out of 12 patients are free of recurrence with an observation time of 2-12 years. Predisposing factors are discussed.

Cancer of the ethmoid is a fairly uncommon disease. According to "Cancer incidence in Sweden" (1968) there are about 70 cancers annually of the nose and paranasal sinuses or about 0.9/100 000 inhabitants. Among these, 10% of the maxilla is in the majority and pure ethmoidal tumours form a minor part. According to Macbeth (1965) cancer of the ethmoid constitutes about 1/3 of all cancers of the nose and paranasal sinuses, and according to Osborn & Winston (1961) about 30%. Frazell & Lewis (1963) report a lower incidence, approximately 10%, and Larsson & Mårtensson (1954) found somewhat less than 5% cancer of the ethmoid among 379 cases of cancer of the paranasal sinuses.

In Sweden the above mentioned figures would mean that the frequency of cancer of the ethmoid would range between 7 and 23 cases every year. It is thus a limited group of tumours and in a small country like Sweden centralization of the treatment is necessary in order to achieve results that are on a level with those presented in

the literature and are based upon a material of cases.

## MATERIAL

We have operated on 12 cases of cancer of the ethmoid with lateral rhinotomy and resection of the anterior skull base after preoperative radiotherapy (Table I). There were 10 males and 2 females and the mean age was 49 years. The mean age in large materials of cancer of the paranasal sinuses is usually somewhat higher, but this material is small and a few patients considerably affect the mean age. The youngest patient was only 17 years old. It seems, however, that the mean age in patients with cancer of the ethmoid is somewhat lower than in a composite material of cancer of the paranasal sinuses. Because of its smallness, the ratio in this material is impossible to give.

## PREDISPOSING FACTORS

A large number of factors predisposing to cancer of the ethmoid have been discussed (Table II). For a long time chronic infections of the nose and paranasal sinuses have been considered

Table I. Cancer of the ethmoid operated on with lateral rhinotomy and resection of the skull base.

No.	Sex	Age
12	10 ♂ 2 ♀	17-68 17-50

2 II Predisposing factors for cancer of the *oid*

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pure III -I dust ure to dust hard wood	Doll 1958 Acheson-Hadfield-Macbeth 1967, Debois, 1969, Gignoux Bernard 1969, Hadfield-Mac- beth 1971
ure to un an factors in and leather stry tional intake nit and nitrat	Acheson-Cowdell Jolles, 1970 Druckrey et al, 1961, 1962 Fong Walsh 1971

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## PATHOLOGY

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Adenocarcinoma is found in a minority of  
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histological types according to the origin of the  
tumour Thus there are very few adenocarcino-  
mas among the tumours of the maxillary antrum,  
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predominance of squamous cell carcinoma and  
adenocarcinomas are more common Thus ac-  
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adenocarcinomas among 40 cancers of the  
ethmoid and Lewis & Castro (1972) found 16  
adenocarcinomas and 47 squamous cell car-  
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In a large material of nearly 400 cases of cancer  
of the nose and paranasal sinuses published in  
1954 by Larsson & Mårtensson, the majority of  
the ethmoidal tumours were adenocarcinomas  
Out of 19 cases there were 11 adenocarcinomas

In our material adenocarcinomas constitute  
50%, or 6 cases out of 12 (Table III) Only one  
case has been classified as squamous cell car-  
cinoma and 3 cases are low differentiated or  
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found among the youngest patients, 17, 27 and  
37 years old It is a moot point whether the  
papillary cylinder cell carcinomas constitute a  
specific histological group of tumours or should  
be assigned to either squamous carcinoma or  
adenocarcinoma, depending on the direction in

Table III Pathology

Type	Number
Adenocarcinoma	6
Low differentiated or anaplastic cancer	3
Papillary carcinoma (Ringertz tumour)	2
Squamous cell carcinoma	1
Total	12

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the literature and are based upon a larger number of cases.

## MATERIAL

We have operated on 12 cases of cancer of the ethmoid with lateral rhinotomy and resection of the anterior skull base after preoperative radiotherapy (Table I). There were 10 males and 2 females and the mean age was 40 years. The mean age in large materials of cancer of the paranasal sinuses is usually somewhat higher, but this material is small and a few patients considerably affect the mean. The youngest patient was only 17 years old. It seems, however, that the mean age in patients with cancer of the ethmoid is somewhat lower than in a composite material of cancer of the paranasal sinuses. Because of its small size, the sex ratio in this material is impossible to evaluate.

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A large number of factors predisposing to cancer of the ethmoid have been discussed (Table II). For a long time chronic infections of the ethmoid and paranasal sinuses have been considered

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Table I *Cancer of the ethmoid operated on with lateral rhinotomy and resection of the skull base*

No	Sex	Age
12	10 ♂ 2 ♀	17-68 M - 50

## VI Postoperative complications

of complication	Number
ive bleeding	1
abscess	1 (?)
sy	1
	3

operative week. Antibiotics were administered prophylactically for about 10 days after operation, mostly as a combination of penicillin and streptomycin (in early cases in this series) and in a few single cases chloramphenicol.

Postoperative complications were few (Table VI). In one case there was profuse arterial bleeding so that we had to ligate the external carotid artery. One case developed a brain abscess four weeks after the operation and died of this complication. One case developed epilepsy which, however, could be controlled without difficulty with antiepileptic drugs. The case with the brain abscess was the only postoperative death. The cause of local recurrence or progression of the tumour—the latter expression ought to be the correct one when we are talking of malignant tumours—three cases were reoperated (Table VII). In these cases we performed a craniotomy and further resection of the tumour of the skull and the dura mater.

## METASTASES

Metastases of cancer of the nose and paranasal sinuses to the regional lymph nodes of the neck are reported in about 15–30% (Larsson & Mårtensson, 1954; Macbeth, 1965; Larsson & Castro, 1972). It seems, however, that metastases are not quite as frequent in cases of cancer of the ethmoid as in those of the antrum

## Table VIII Metastasis

Twelve cases of cancer of the ethmoid

Cervical lymph nodes	1
Distant metastasis	—

(Larsson & Mårtensson, 1954). Out of our 12 cases only one developed metastases in the regional lymph nodes in the neck (Table VIII). In one other case neck dissection was performed on a strong clinical suspicion of metastases, but this could not be verified at the histological examination. Prophylactic neck dissection was not performed.

## RESULTS

Of 12 cases of cancer reviewed above 8 are alive without signs of tumour. Two have died of their disease. One died postoperatively and one of intercurrent disease 2 years after the operation (Table IX). At autopsy the patient who died of a brain abscess 4 weeks after the operation as well as the one who died of intercurrent disease after 2 years were found to be free of cancer and without metastases in the regional lymph nodes or in more distant locations. If the patient who died postoperatively is considered dead of cancer, 9 cases are still alive. The survival time varies but 7 out of these 9 cases have been alive for 6–12 years (Table X).

## DISCUSSION

Cancer of the ethmoid is a fairly unusual tumour, at least in its pure form. In the individual case with progression of the tumour to several sinuses it is always difficult to decide whether the origin was the ethmoidal cells or whether the tumour started in the maxillary antrum and then invaded

## Table VII Reoperation for local recurrence

of operation	Number	Alive
Craniotomy with further resection of the skull base and the dura	3	2

## Table IX Results

Alive without signs of tumour	8
Died of cancer	2
Died postoperatively	1
Died of intercurrent disease	1
Total	12



Table X *Observation time in cases alive and without signs of recurrence*

Number of patients	Years
2	12
1	11
1	7
2	6
1	2
1	1.5

the ethmoid area. In the literature, opinions differ as to the area where the tumour arises and this uncertainty is reflected in widely varying frequency figures. According to some authors pure ethmoidal cancer is extremely rare, but according to others cancer usually starts in the ethmoid and especially in the so called antro-ethmoidal cells, invading the maxillary antrum later (Macbeth, 1965).

As regards predisposing factors there seems to be statistical evidence that morbidity in cancer of the ethmoid is higher among people exposed to certain industrial air pollution but the specific carcinogenic substances have not been found or chemically isolated. In addition to chemically active carcinogenic substances it is possible that unspecific irritation by air pollution may play a

certain role. Snuff-taking has also been mentioned as an instrumental factor, but this is doubtful and a higher morbidity among people using snuff has not actually been demonstrated. Nor is there any certain connection between tobacco smoking and cancer of the ethmoid (Hadfield & Macbeth, 1971). As we know that the main part of the air inhaled through the nose passes through the middle and upper nasal areas it is reasonable to assume that the local carcinogenic substances will act mainly in the ethmoid area. There are no reports indicating that exposure to air pollution contributes to induce cancer of the nose and paranasal sinuses elsewhere than in the ethmoid cells. It has been discussed, however, if not recurrent or chronic infections in the nose and sinuses may be of a certain importance for the origin of all forms of

cancer of the nose and paranasal sinuses. Also nasal polyps and squamous cell carcinoma are said to be significantly higher. Authors who hold this view have shown that up to 4% of cases have been surgically treated for allergic diseases within nose and paranasal sinuses (Lewis & Castro, 1972). This is less than the statistical mean in a normal population (Harrison (1973), however, strongly doubts this correlation. With the exception of a morbidity in certain areas owing to certain substances in industrial work (Baker, 1964, for example), cancer of the ethmoid seems to exist in about the same frequency as other

The ratio men-women varies in different reports. Larsson & Mårtensson (1964) do not find any clear sex dominance while Lewis & Castro (1972) find a predominance of men in the relation 2 to 1. The ratio for ethmoid cancer seems, however, to differ from that of cancer of the antrum so that men are more strongly affected. This is perhaps reasonable in view of the different carcinogenic substances that have been found in different factories. In spite of the Women's Liberation Movement still predominate in the industries in which is likely to affect the sex ratio.

As to the treatment, aggressive surgery and therapy combined with radiotherapy are the best possibilities of curing the patient. If the tumour has invaded the anterior base of the skull and the dura mater, good results can be achieved by resection of the areas involved. A combined approach with frontal craniotomy and rhinotomy or the Dencker operation has been described by among others Gumpert & Kleitsch (1967), Keitcham et al. (1967), Bridger & Shaheen (1968). We have found these excessive operations necessary and the resection of the anterior base of the skull and the dura mater can be done without difficulty by a combined rhinotomy. In our opinion cooperation with a neurosurgeon is of the greatest importance in this situation, since the otosurgeon does not enter the skull cavity with the same ease as a surgeon trained in neurosurgery. It is our opinion that such team work provides the best

to cure the patient and leads to better results as a whole

Postoperative complications have been relatively few in spite of the fact that the area of surgery must be considered highly contaminated although meninges and brain have been laid open. This is probably due to the heavy antibiotic therapy administered as a routine. In the antibiotic era meningitis should probably not have been a much more common complication. In our experience of the resorbable material used the results are very good. It quickly induces fibrosis which effectively prevents cerebrospinal fluid fistulas. A new 'base of the skull' made up of connective tissue was also found to be very strong. In the three cases reoperated on with further resection because of recurrent or residual tumour it is also somewhat surprising that only one case developed postoperative epilepsy, especially since in some patients the dura had been resected and the brain surface covered with a hard and resorbable material or by resorbable material only. The absence of this complication is probably due to the fact that no brain substance was removed and that there was thus no focus for scars of the epileptogenic type.

It is well known in cancer surgery that chances of success are limited in the case of reoperation for recurrent or residual tumour. The patient's life lies in the primary operation which must be made radical. In our material there are, however, three cases reoperated on for local recurrence, and two of these are still alive with a very long observation time without signs of residual tumour or metastases. This we think justifies the fact that the prognosis of ethmoid cancer is somewhat better than that of cancer of the maxillary antrum, mainly owing to its tendency to produce metastases but to recur locally in a way which admits of successful surgical treatment. It seems likely that especially adenocarcinomas of the ethmoid area develop neck metastases less frequently than tumours of the maxillary antrum (Larsson & Mårtensson, 1954). It is also possible that the better prognosis of cancer of the ethmoid is due to its tendency to give symptoms early in contrast to cancer of the

maxillary antrum, where symptoms often appear only when the bony walls have been destroyed by the tumour (Harrison, 1973).

According to our experience tumours invading bone can be favourably affected by radiotherapy if the tumour is radiosensitive, but only in exceptional cases can all the tumour cells be killed and the patient cured. As we know that diminished vascular supply and lowered oxygen tension in the tissues decrease the sensitivity of the tumour to radiotherapy it is also reasonable to provide the main part of the treatment preoperatively.

It might seem irrational to provide preoperative radiotherapy in all cases of cancer of the ethmoid without regard to the histological type. Adenocarcinoma is as a rule less sensitive to radiotherapy. Judging from the histological type it is however impossible in the individual case to decide if the tumour is going to respond to treatment or not. Many adenocarcinomas react very well to radiotherapy and some of our best results were obtained in cases of adenocarcinoma. We therefore find it justified to give preoperative radiotherapy as a matter of routine without regard to the histological type.

In no case has the eye been sacrificed. The capsula tenoni seems to be very resistant to tumours and only in one case did we have good reason, judging from the growth of the tumour, to exenterate the orbit. In this particular case the patient did not allow us to remove the eye because he was blind on the other side. Yet the rule must be that if there is the slightest sign or suspicion of tumour invading the orbit, this should be exenterated. Some surgeons claim that the eye should be removed as a matter of routine because it is injured by the radiation and of little use to the patient. Since, however, modern technique in radiotherapy allows effective shielding of the eye it is unnecessary to exenterate the orbit merely because of the risk of radiation damage to the eye.

This material is of course too small to allow any certain conclusions as regards prognosis with the combined therapy described above. We think, however, that the results tend to show

better figures than the overall figures for cancer of the nose and paranasal sinuses, and that thus further use of this combined radiological-otological-neurosurgical technique is indicated

## ZUSAMMENFASSUNG

Eine Patientengruppe mit 12 Fällen von Karzinom des Ethmoidalsinus wird beschrieben. Die Behandlung war eine Kombination von Radiotherapie und Chirurgie mit pre- oder eventuell postoperativer Strahlenbehandlung und lateraler Rhinotomie mit Resektion der vorderen

von zwölf Fällen leben ohne Rezidiv mit einer Beobachtungszeit von 2 bis 12 Jahren. Predisponierende Faktoren werden diskutiert.

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## HISTOCHEMISTRY OF PRIMATE LARYNGEAL MUSCLES

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(Received January 22, 1974)

The intrinsic laryngeal muscles of adult anesthetized rhesus monkeys were removed by microsurgical technique and studied for the following histochemical reactions: ATPase with and without acid preincubation, NADH, LDH, SDH and PAS. In the abductor, posterior cricoarytenoid, 60% of the fibers had acid labile ATPase activity and 40% of the fibers exhibited a high content of oxidative enzymes. The muscle of the vocal folds, cricothyroid, showed a mixture of acid labile fibers (70%) and gave a weakly strong reaction for oxidative enzymes. The cricoarytenoid muscle contained 81% acid labile and acid-resistant fibers. The lateral cricoarytenoid and cricothyroid muscles were almost exclusively composed of acid labile fibers and showed a typical checkerboard pattern of oxidative enzymes.

Laryngeal muscles are functionally very fast (Lund, 1969; Mårtensson & Skoglund, 1964). Lund & Conesina (1965) demonstrated that like the lateral rectus muscle of the orbit, the laryngeal muscles showed the presence of up to 70% of muscle fibers with multi motor endplates. The percentage distribution of multi motor end plate fibers in a muscle bore a direct relationship to its contraction time. The ultrastructure and enzyme contents of laryngeal muscles have been reported by several authors (Berendes & Vogel, 1960; Ganz, 1971). Lund (1968), Hall Craggs, for the first time, reported on correlating the contraction times and histochemical picture of laryngeal muscles of the rabbit. Syrový & Gutmann (1971), employing

biochemical techniques, found a direct relationship between the quantity of myosin ATPase and the speed of contraction of the thyroarytenoid and cricothyroid muscles of the rabbit.

In this report we present the histochemical characteristics of the intrinsic laryngeal muscles of the rhesus monkey (*macaca mulatta*) thyroarytenoid, cricothyroid, posterior cricoarytenoid, lateral cricoarytenoid and interarytenoid. An analysis of the histochemical profile of these very fast muscles is essential to an understanding of the biochemical nature of their motor units. The findings of this study indicate that the majority of motor units of the intrinsic laryngeal muscles are composed of a unique fiber type that is both fast-contracting and fatigue resistant.

### MATERIAL AND METHODS

Intrinsic laryngeal muscles of adult rhesus monkeys (*macaca mulatta*), anesthetized with pentobarbital sodium, 25 mg/kg, were removed by microsurgical technique. The muscle tissue obtained was quickly frozen in isopentane and liquid nitrogen. Serial, but not contiguous, 10-12  $\mu$ m sections cut in a cryostat were studied for the following histochemical reactions.

**ATPase.** Myofibrillary ATPase activity was determined by the method of Padykula & Herman (1955) at pH 9.4. In this system, two main fiber types were distinguished: light staining, low ATPase activity and dark staining, high ATPase

This study was supported in part by U.S. Public Health Service Grant NS-09723 and SRS Grant 16-P-56809-5-05.

better figures than the overall figures for cancer of the nose and paranasal sinuses, and that thus further use of this combined radiological-otological-neurosurgical technique is indicated

## ZUSAMMENFASSUNG

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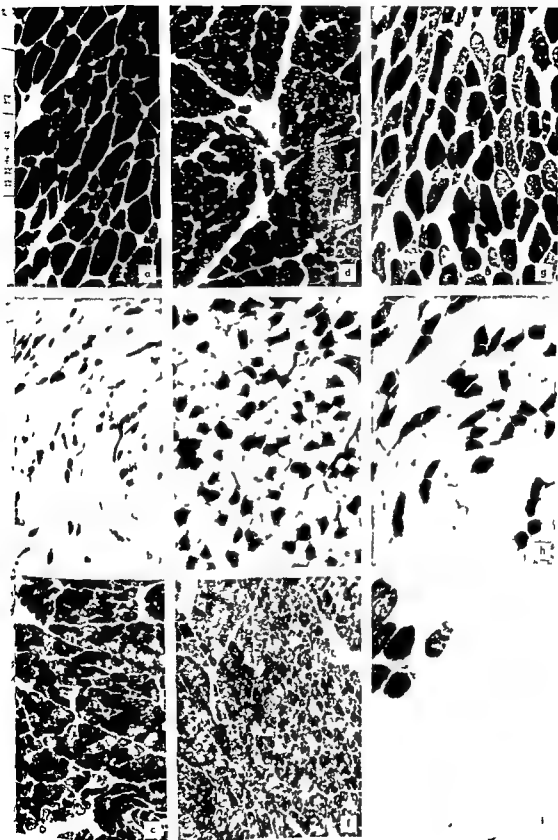


Table II Comparison of acid-labile and acid resistant fibers (by ATPase reaction) of laryngeal muscles

Muscle	Contraction time	% Acid resistant (%)	% Acid labile (%)	Acid resistant / Acid labile
Vastus medialis	50 msec (human) <sup>a</sup>	38	62	0.61
Thyroarytenoid	14 msec (primate) <sup>b</sup>	19	81	0.23
Cricothyroid	36 msec (primate) <sup>b</sup>	30	70	0.43
Posterior cricoarytenoid	44 msec (dog)	40	60	0.67
Lateral cricoarytenoid	19 msec (dog)	12	88	0.14

<sup>a</sup> Brooke & Engel (1969)<sup>b</sup> Hast (1969)

Previous research has shown that the speed of contraction of canine laryngeal muscle is similar to that of monkey. See Hast M. H. 1967 *Ann Otol* 76: 489. Hast, M. H. & Golbus S. 1971 *Proctorel* 10: 100.

Acid preincubation results in a loss of myofibrillary ATPase activity in about 60% of the muscle fibers. The ratio of acid resistant to acid labile fibers was 0.66 (Fig 1g, h).

**Lateral cricoarytenoid muscle** in this muscle, a very large majority of muscle fibers showed strong actomyosin ATPase reaction. Acid preincubation, however, resulted in a loss of myofibrillary activity in the majority of muscle fibers. The ratio of acid resistant to acid labile fibers was 0.14.

**Interarytenoid muscle** in this muscle, a large majority of the muscle fibers also showed high actomyosin ATPase activity. With acid preincubation the majority of muscle fibers lost their myofibrillary ATPase activity.

## DISCUSSION

The speed of muscle contraction has been directly correlated with the concentration and pH characteristics of actomyosin ATPase activity (Barany, 1967; Guth & Samaha, 1969; Brooke & Engel, 1969). In the gastrocnemius muscle of the cat, fast twitch motor units (contraction time 16–30 msec) have been shown to have high ATPase activity which is acid labile and alkali-stable (Burke, 1967; Yellin & Guth 1970; Burke et al., 1971).

Among the laryngeal muscles the thyroarytenoid and lateral cricoarytenoid are the fastest

(contraction times 14 and 19 msec) and have high ATPase activity and a preponderance of fibers with acid labile myosin ATPase. The corresponding oxidative enzymes, however, exhibit a more or less uniform distribution indicating that these muscle fibers have a high oxidative metabolism.

The cricothyroid (vocal fold tensor) and posterior crico-arytenoid (the glottal abductor) muscles have contraction times of 36 and 44 msec, respectively. Their ATPase profiles show a mixture of dark and light reacting fibers, the dark fibers being acid labile. Such a pattern has been observed in the human cricothyroid. The oxidative enzyme pattern in the cricothyroid shows a mixture of intermediate and fast reacting fibers, while in the posterior crico-arytenoid the pattern is more homogeneous. This distribution is reminiscent of the mixed fiber type.

In conclusion, the faster contracting laryngeal muscles are rich in ATPase activity and have a higher distribution of acid labile ATPase. The ratio of acid resistant ATPase activity is directly related to the speed of contraction. The oxidative enzyme distribution pattern shows that all the laryngeal muscles have high activity. Similar patterns have been observed in the extraocular muscles (Tenenbaum et al., 1972) and in the tensor tympani and pectoralis muscles of the cat (Tenenbaum & D.

## ZUSAMMENFASSUNG

Inneren laryngealen Muskeln von erwachsenen Rhesusaffen wurden durch eine mikroskopische Technik ertötet und folgende histochemischen Reaktionen darin untersucht: ATPase mit und ohne Vorbehandlung bei pH 4.4, NADH, LDH, SDH AS. In dem Abduktor, musculus cricoarytenoideus, hatten 60% der Fasern eine hohe saure labile Aktivität, 40% der Fasern zeigten eine saure ATPase-Aktivität, diese Muskelfasern zeigten einen hohen Gehalt an oxydativen Enzymen. In der Stimmbänder (musculus cricothyroideus) eine überwiegende Menge von saure labilen Fasern und gab eine gleichmässig starke Reaktion für diese Enzyme. Der thyroarytenoide Muskel bestand aus saure labilen und 19% saure resistenten Fasern. Interarytenoide und interarytenoide Fasern waren fast ausschliesslich aus Fasern mit labilen Enzymen zusammengesetzt und besaßen ein typisches Streifenmuster von oxydativen Enzymen.

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# ENZYME HISTOCHEMISTRY OF SQUAMOUS CELL CARCINOMA OF THE LARYNX

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**Abstract** Based on the clinical observation that the prognosis of larynx carcinoma is much more favorable in the female than in the male, biopsy specimens from 36 untreated patients (32 males and 4 females) were studied in an attempt to determine whether this apparently sex linked divergence in tumour behaviour is also reflected in the enzymic histochemical pattern characterizing these tumours. From this material it is apparent that the enzyme pattern characteristic of squamous cell carcinoma of the larynx closely resembles that of analogous tumours with other localizations. In addition the activities of various enzymes—although showing a great deal of individual variation—correlate with the degree of morphologic differentiation of the tumour. Finally it is concluded from the relatively scanty material at our disposal that there are neither clear-cut nor consistent differences in the histochemical pattern of larynx carcinoma according to sex of the host.

It is a well-established clinical fact that the prognosis of laryngeal carcinoma is much more favorable in the female than in the male (Kirchner & Malin 1953, Piquet, 1958). The present study was performed in an attempt to determine whether this apparently sex linked divergence in tumour behaviour is also reflected in the enzymic histochemical pattern characterizing these tumours.

## MATERIAL AND METHODS

The material consisted of biopsy specimens from 36 untreated patients (32 males and 4 females) which contained adequate amounts of neoplastic tissue without a degree of infection or necrosis that could possibly interfere with the correct

interpretation of the enzyme content of neoplastic epithelial cells.

The tumours were graded as follows: moderately differentiated (Grade II) or poorly differentiated (Grade III) carcinoma. The incidence of tumours of various grades is given in Table I.

Punch biopsy samples were quickly frozen in carbon dioxide, serially cut on a microtome, and processed to demonstrate the activity of the following enzymes: alkaline phosphatase, 5-nucleotidase, acid phosphatase, non specific esterase (using naphthol AS D acetate and  $\alpha$  naphthyl acetate), leucyl aminopeptidase, succinic dehydrogenase, lactic dehydrogenase,  $\beta$ -hydroxy-butyric dehydrogenase, malic dehydrogenase, glucose 6-phosphatase, aspartate aminotransferase,  $\alpha$ -glycerophosphate dehydrogenase, and uridine diphosphoglucose dehydrogenase. The techniques applied have been described previously (Thiery, 1963, Willighagen et al 1973). The demonstration of succinic dehydrogenase activity on the slides was pretreated with PMS.

Several frozen sections fixed in Bouin's fluid and dehydrated were stained with hematoxylin-eosin, periodic acid schiff (PAS, with diastase control), and Oil red O.

## RESULTS

The activity range of some of the enzymes and the distribution of the

## I Incidence of tumour grades in the present according to sex

Squamous cell carcinoma of	n cases	
	Male	Female
I	18	2
II	11 <sup>a</sup>	1
III	3 (5)	1
	32	4

<sup>a</sup> Of these tumours also contained appreciable areas of grade III squamous cell carcinoma. Thus, the number of squamous cell carcinomas of the larynx studied in the male is 35, as indicated within parentheses.

neoplastic squamous cells are given in Tables II and III, for the male and female patients, respectively. Tumours were classified according to the grade of morphologic differentiation.

## Squamous cell carcinoma in male patients

Larger quantities of glycogen are found more often in differentiated than in the more anaplastic tumours. As noted in squamous cell carcinoma in general, PAS-positive material tends to be distributed in the keratinizing neoplastic cells.

## II Range of activity of certain enzymes and distribution of glycogen in squamous cell carcinoma of larynx in males

	PAS	Al	ZI	5N	$\alpha$ -est
Grade I					
1	0 +	0	+	0	-
2	0 +	0	-	0	- - -
3	0 +	0	++	0	-
4	0 -	0 loc ±	- -	0 ±	- -
5	0	0	+	0	++
6	0 loc -	+	+	0	0
7	0 +	0	++	0	0
8	0 loc - - +	0	++	0	- -
9	0 -	0	+/ -	0	-
10	0 + +	0	++ / +	0	- -
11	0 ±	0	++	0	- -
12	0	0	+	0 loc ±	-
13	0 loc -	0	+	0	- - -
14	0 -	0	0 +	0	- -
15	0	0	+	0 -	-
16	0 -	0	+	0	-
17	0	0	+	0	-
18	0 + +	0	+	0	-
Grade II					
19	0	0	- -	0	- -
20	0	0	- -	0	- -
21	0	- { + +	+	0	-
22	0 -	0 loc ±	+	±	± - -
23	0 loc +	0	++	0	- -
24	0 loc +	0	++	0	- -
25	0	0	- -	0	-
26	0	0	+/ +	0 loc -	- / +
27	0	0	+	0 ±	-
28	0	0 loc + +	- / -	0	-
29	0	0	-	0	+
Grade III					
30	0	-	-	0	0
31	0 loc ±	0	-	0	- / - -
32	0	+	-	+	-
33	0	0	+	0	-
34	0	0 ±	-	- -	0 loc -

<sup>a</sup> Tumours also containing areas of grade III squamous cell carcinoma. PAS = glycogen; Al = alkaline phosphatase; 5N = 5 nucleotidase;  $\alpha$ -est =  $\alpha$ -naphthyl esterase; 0 = no enzyme activity; ± = trace activity; + = weak activity; ++ = moderate activity; +++ = high activity; - - - = very high activity; loc = local activity.

Table III Activity range of certain enzymes and distribution of glycogen in squamous cell carcinoma of the larynx in females

Tumour grade	Case no	PAS	AI	ZI	5N	α-GI
I	33	0 +	0	++/+++	0	+/++
	34	0 loc +	0	++	0	+/++
II	35	0	0	+	0	+
III	36	0 loc +	0	+	+	/

Key: see Table II

Larynx carcinoma is usually void of alkaline phosphatase activity, but with progressive dedifferentiation the squamous cells more often display some histochemically demonstrable activity of this enzyme. The same trend is known to exist in carcinoma of both the uterine cervix (Thiery & Willighagen 1966) and the oesophagus (Willighagen unpubl.).

In carcinoma of the larynx the activity of acid phosphatase varies considerably. A definite relationship with the degree of morphologic differentiation is not conspicuous but in one of the present tumours (no 25) containing two clones of neoplastic cells acid phosphatase activity was clearly lower in the anaplastic tumour population.

Although only weak (or even absent) activity of 5 nucleotidase is demonstrable in the neoplastic squamous cells. The enzymic content of these cells increases with dedifferentiation phenomenon also displayed by one of the tumours (no 25) which contained a double cell population.

Tumour cells show divergent activities of non-specific esterases which are most clearly shown with  $\alpha$ -naphthyl acetate as substrate and range from 0 to ++.

It is our contention that the mean activity correlates with tumour grade being highest in Grade I tumours. This phenomenon is understandable because in squamous cell carcinoma of the uterine cervix (Thiery & Willighagen 1966) and the lung (Willighagen et al 1963) non-specific esterase activity coincides with keratin formation.

In contradistinction with the findings in

cervical carcinoma (Thiery & Willighagen 1966) the overall activity of lactic dehydrogenase in squamous cell carcinoma of the larynx decreases with differentiation. This however is illogical if it is taken into consideration that basal and parabasal neoplastic cells show the most intensive staining, while keratin pearls are almost devoid of demonstrable activity. The activity of  $\alpha$ -glucose dehydrogenase on the other hand seems to be correlated with the degree of morphologic differentiation. In larynx carcinoma the relative intensity of the dehydrogenase studied is essentially similar to that of malignant tumours in general (Willighagen 1963) and can be summarized as follows: lactic dehydrogenase > succinic dehydrogenase > PMS >  $\alpha$ -glycerophosphate dehydrogenase > OH-butyric dehydrogenase > glucose-6-phosphate dehydrogenase > indoxyl phosphate dehydrogenase.

No cellular activity of adenonucleotidase was found in larynx carcinoma except in Grade II tumour (no 25) where the enzyme was clearly indicated by the strong red reaction.

In some of the Grade I tumours ATP positive (Langerhans) cells were found. These elements are characteristic of basal and pathologic squamous epithelium (Willighagen 1969a and b).

Although no aminopeptidase activity was present in the neoplastic epithelium, the connective tissue surrounding neoplasia invariably showed activity of this enzyme, unrelated to the degree of tumour differentiation.

IV *Acetate esterase activity and distribution of glycogen and lipids in squamous cell carcinoma of larynx according to tumour grade*

Enzyme	Tumour grade		
	I	II	III
alkaline phosphatase	0	0	+
acid phosphatase	0	0	+
leucodase	0	0	+
nucleoside phosphatase	0	0	0
ethyl esterases	+	+	+
aminoesterase	0	0	0
dehydrogenase		+	
phosphatase	+		
dehydrogenase	0	0	0
gen	0	0	0

is could not be demonstrated in neoplastic except in necrotic areas

*squamous cell carcinoma in women*

In the scanty of laryngeal carcinoma in the female the number of lesions studied is small does not permit the drawing of definite conclusions. The available data strongly suggest however that roughly the same correlation between tumour grade and enzyme activities and distribution of glycogen and lipids is found in carcinoma affecting men and women. Further there is a clear-cut difference between the sexes with respect to the histochemically demonstrable glycogen content of the tumour seems improbable.

## CONCLUSIONS

In this selected series of biopsy specimens it is apparent that the enzyme pattern characterizing squamous cell carcinoma of the larynx closely resembles that of analogous tumours at other localizations, for example the uterine cervix (Thry & Willighagen 1966). In addition the activities of various enzymes—although showing a great deal of individual variation—correlate with the degree of morphologic differentiation of the tumour. This is clearly shown in Table IV which gives a highly simplified

presentation of the histochemical profile. Finally it is concluded from the relative scanty material at our disposal that there are neither clear-cut nor consistent differences in the histochemical pattern of laryngeal carcinoma according to sex of the host.

## SUMMARY

1 The activity of a number of hydrolytic enzymes and dehydrogenases and the distribution of glycogen and lipids were studied in 36 cases of laryngeal carcinoma (32 males and 4 females).

2 The enzyme histochemical pattern characterizing this tumour closely resembles that of squamous cell carcinoma of other origin.

3 A definite correlation between tumour grade and enzyme pattern was found to exist. With higher degrees of differentiation tumour cells tend to show higher activities of alkaline phosphatase, 5-nucleotidase and lactic dehydrogenase, whereas the reverse holds for non-specific esterases. The glycogen content is highest in the differentiated neoplastic squamous cells.

4 The sex-linked difference in biological behaviour characteristic of laryngeal carcinoma was not found to be clearly reflected in the histochemical pattern characterizing these tumours.

## ZUSAMMENFASSUNG

Aufgrund der klinischen Beobachtung, dass die Prognose des Kehlkopf-Carcinoms bei Frauen viel günstiger ist als bei Männern, untersuchten wir 36 Biopsiematerialien von 32 männlichen und 4 weiblichen Patienten. Wir versuchten, festzustellen, ob dieser offensichtliche geschlechtsgebundene Unterschied im Tumorverhalten sich auch im histochemischen Enzymmuster widerspiegelt, das diese Tumoren charakterisiert. Aus diesem Material ist ersichtlich, dass das für das Plattenepithel-Carcinom des Kehlkopfs charakteristische Enzymmuster sehr grosse Ähnlichkeit mit dem analoger Tumoren anderer Lokalisation aufweist. Ausserdem korrelieren die verschiedenen Enzymaktivitäten—trotz grosser individueller Schwankungen—with dem Grad der morphologischen Differenzierung des Tumors. Schliesslich wird aus dem relativ kleinen uns zur Verfügung stehenden Material geschlossen, dass weder scharf begrenzte noch einheitliche Unterschiede im histochemischen Enzymmuster des Kehlkopf-Carcinoms in bezug auf das Geschlecht des Carcinomtragers bestehen.

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# LUNG FUNCTION BEFORE AND AFTER LARYNGECTOMY

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(Received January 7, 1974)

Five patients were examined with respect to airway mechanics before and after laryngectomy. Methods were used, the results of which were not affected by the laryngeal stenosis before and by any stenosis of the tracheostoma after the operation. It was found that the pulmonary resistance from trachea to lungs was normal before operation. Postoperatively pulmonary resistance increased and reached a maximum six months, when it was about ten times as high as preoperatively. One year after operation it was not more than about twice the original value. At that time lung function was normal as concerns working capacity and arterial blood gases. A great adaptability of the airways was shown. The dangers and problems during the postoperative period are discussed.

During the postoperative period laryngectomized patients often suffer from respiratory symptoms due to the elimination of the air-conditioning function of the upper airway. The lung function before and after laryngectomy has been studied by others (Heyden, 1950, Fourage, 1964), but interpretation of the results has been complicated by the influence of the varying degree of stenosis caused by the cancer and the tracheostoma.

In order to avoid such influences we have restricted our determinations to such measures as are not affected by the upper airways. We have, for example, determined the resistance of the trachea and airways from the pleura up to the tracheostoma. In contrast to earlier investigations we have followed the lung function with repeated measurements until one year after operation.

This study was supported by the Swedish Medical Research Council against Heart and Chest Disease and by the Swedish Medical Research Council grant no. B-7214X.

## MATERIAL

Ten consecutive patients undergoing laryngectomy, September-December 1970, because of laryngeal cancer, were studied before and after operation. Five of these patients could not be examined as planned, for the following reasons: (1) postoperative death, (2) pulmonary metastasis (the only woman), (3) mental disease, (4) uraemia, and (5) local infection around the tracheostoma. The patients who completed the investigations were 5 men, with a mean age of 64 years (range 55-75), all of them with a history of regular smoking for 15-30 years. As to age and smoking habits they are typical patients with laryngeal cancer. The oldest subject (no. 1) had a history of pulmonary tuberculosis 9 years before operation. Repeated bacterial controls had since then been negative. Roentgenological and spirometric investigation showed severe permanent damage to the lungs. The patient suffered from exercise dyspnoea and from angina pectoris since a myocardial infarction in 1965.

One patient (no. 4) suffered from chronic bronchitis. He had for several years had a cough with expectoration but had never sought medical advice. The remaining 3 subjects had no history of lung disease. A chest X-ray was performed at each examination. There were no roentgenological signs of parenchymal disease except for those observed in patient 1. No changes were observed during the postoperative period.

Table I Age and static lung volumes

Total lung capacity, TLC, residual volume RV, vital capacity, VC, and functional residual capacity FRC, are measured values in percent of values predicted according to Grimby and Söderholm (1961)  
w = weeks and m = months after operation

Subject no	Age years	TLC, % of predicted value					RV, % of predicted value				
		Pre op	2 w	2 m	6 m	12 m	Pre-op	2 w	2 m	6 m	12 m
1	75	103	79		100	97	183	117		154	
2	63	86		87	108	97	84		116	108	
3	62	104	88	89	102	106	137	113	104	113	
4	65	108	97	107	113	114	125	129	167	131	
5	55	125	101	110	116	107	145	127	132	114	
Means	64	105	91	98	108	104	131	122	130	134	

## METHODS

Each patient was examined before, and 2 weeks, 2, 6, and 12 months after laryngectomy. Pulmonary resistance, static elastic pressure volume curve and static lung volumes were determined. Twelve months after laryngectomy a test was performed of the working capacity on a bicycle ergometer. During this test arterial blood gases were examined as well as the resistive work rate of breathing, the functional pulmonary resistance and the breathing pattern.

### Pulmonary resistance and static elastic pressure volume curve

Pulmonary resistance and the static pulmonary pressure volume curve were measured with the flow regulator method (Jonson, 1969). This method allows determination of pulmonary resistance under standardized conditions with regard to breathing pattern and flow rate. Resistance was in the present investigation measured at a flow rate of 1 l/s throughout long expirations starting at lung volumes close to the total lung capacity. Resistance was related to the static recoil pressure of the lungs  $P_{st}(l)$ . This was done as  $P_{st}(l)$  is a very important determinant of resistance (Fry, 1958; Butler et al., 1960; Jonson, 1969, 1970a, b; Stubbs & Hyatt, 1972; Colebatch et al., 1973). When resistance determinations are made for the purpose of revealing pathological changes within the bronchi the

values of resistance must consequently be obtained at given values of  $P_{st}(l)$  (1) and (2). In the present study values of  $P_{st}(l)$  refer to expirations at 1 l/s and a  $P_{st}(l)$  of  $H_2O$ , if not otherwise stated.

Only those details which differ from the original method (Jonson, 1969) are brought into further consideration.

The pressure difference between oesophagus and mouth includes the pressure drop in extrathoracic airways. It can therefore not be used in the present investigation where the influence of these airways must be eliminated. We have measured the pressure difference between oesophagus and mouth between oesophagus and trachea in order to determine the pulmonary intrathoracic resistance. The pressure in the oesophagus was measured via a balloon (Jonson, 1969) and the pressure in the trachea via a polyethylene catheter with a hole close to the closed tip. The catheter was introduced under local anaesthesia and direct laryngoscopy at the preoperative examination. Postoperatively a tracheal cannula (no. FH36) was inserted and used to avoid leakage. The tracheal cannula pressure recordings was passed down the cannula so that its tip was about 2 cm above the carina. The same procedure for the catheter tip was aimed at pre- and postoperatively. This was achieved by the known position of the tip relative to the larynx.

of predicted				FRC, % of predicted value				
2 w	2 m	6 m	12 m	Pre-op	2 w	2 m	6 m	12 m
62		81	67	124	87		129	126
63	83	113	96	83		78	142	100
67	78	82	88	125	94	92	125	122
84	76	96	96	131	106	155	139	145
87	100	104	92	129	129	140	151	143
73	84	95	88	118	104	117	137	127

subject the estimation was checked by and found to be adequate

pressure in oesophagus and trachea was by separate transducers (EMT 34,

Elema AB) and the difference calculated by an electronic subtraction unit

*Static lung volumes* Total lung capacity, TLC, residual volume, RV, vital capacity, VC, and functional residual capacity, FRC, were determined with a body plethysmograph (Jonson,

*Exercise and lung function test* One year after laryngectomy a graded exercise test on a bicycle was performed. During this test arterial blood samples were drawn and analysed with

(Eschweiler & Co) with respect to partial pressure of oxygen,  $P_{aO_2}$ , and carbon dioxide,  $P_{aCO_2}$ . Pulmonary functional resistance, and minute ventilation were measured at a load of 1 l in the viscous resistance of the lungs including the extrathoracic airway at a spontaneous breathing pattern during exercise (Jonson & Jonson, 1973)

*Preoperative test* An air-tight soft mask was fitted around the tracheostoma for the periods required for measurement of pulmonary mechanics. The mask, which did not occlude the tracheostoma, connected the latter to a pneumotachograph. The flow signal from the pneumotachograph and the signal representing the pressure were fed to the computer (PDP-8, Digital Equipment) which per-

formed the calculations (Jansson & Jonson, 1973). The load on the bicycle ergometer was stepwise increased until the patient was exhausted.

Subjects 1, 3, 4 and 5 were given 0.5 mg atropine subcutaneously 1 hour before each investigation. This was done as the first patient (no. 2) showed an increased rate of secretion which caused discomfort and technical problems during the measurements. This subject was not given atropine even at the later investigations, in order to allow for comparisons of data. All subjects were anaesthetized with a spray of 1–2 ml of Xylocain® (Lidocain) into larynx or trachea.

## RESULTS

The preoperative study showed a somewhat poor elastic recoil of the lungs at low volumes in all patients (Fig. 1). FRC and RV were higher than expected. TLC was normal and VC accordingly on the average slightly lower than expected (Table 1).

The static elastic pressure-volume diagram did not change after operation, nor did RV. Two weeks and 2 months after operation FRC was slightly lower than before, but increased later to values just above those observed preoperatively. TLC and accordingly VC decreased considerably after operation, but increased later to values close to those observed preoperatively.

Resistance measured from pleural pressure to mouth (RI) before operation



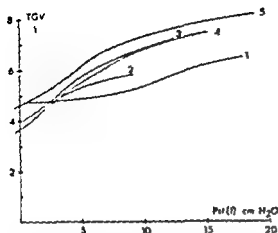


Fig 1 The elastic recoil pressure of the lungs was lower than in normal subjects (shadowed area), especially in the low volume range of each patient  $Pst(l)$  is recoil pressure, TGV = thoracic gas volume

normal in subjects 1, 3 and 5 (Table II, normal range 1–3 cm  $H_2O/(l/s)$ ). In the same subjects the intrathoracic resistance  $R_{lth}(l)$ , was, on average, 5.4 cm  $H_2O/(l/s)$  lower (Table II). This difference corresponds to the resistance of the airways from the tracheal catheter tip to the mouthpiece. It is much higher than normal values (roughly 1 cm  $H_2O/(l/s)$ ) (Ferris et al, 1964). The high value can be attributed to the stenosis caused by the tumour. Subjects 2 and 4 showed normal values of resistance in spite of the tumour. According to the data of Ferris et al (1964) normal values of  $R_{lth}(l)$  can be estimated to range between 0.3 and 1 cm  $H_2O/(l/s)$ . All subjects except no. 1 had normal values of  $R_{lth}(l)$  before operation. In subject 1 the value is explained by his lung disease.

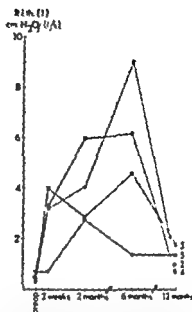


Fig 2 The resistance of the intrathoracic airways  $R_{lth}(l)$ , was normal before operation, but reached a maximum about 6 months after operation and then turned towards normal values.

After operation  $R_{lth}(l)$  increased in all subjects (Table II and Fig. 2). In subject 1  $R_{lth}(l)$  had increased more than 10 times 6 months after operation. After 12 months  $R_{lth}(l)$  had fallen to values about twice as high as before operation.

In order to find out if the high values of resistance observed 2–6 months after operation were due to bronchospasm, determinations of  $R_{lth}(l)$  were repeated in 3 patients after administration of 0.5 mg adrenalin subcutaneously. In subjects 1 and 2 no change of  $R_{lth}(l)$  was observed. In subject no. 4 (6 months after operation)

Table II Resistance before and after operation

$R_l$  is resistance including the extrathoracic airway before operation.  $R_{lth}(l)$  is resistance from pleura to trachea.  $R_f(l)$  is the functional resistance during exercise (Jansson & Jonson, 1973) including the resistance of the extrathoracic airway.

Subject no	Rl cm H <sub>2</sub> O (l/s) Preop	Rl th(l) cm H <sub>2</sub> O (l/s)					Rf(l) cm H <sub>2</sub> O (l/s) 12 months
		Preop	After operation				
			2 weeks	2 months	6 months	12 months	
1	13.1	5.0	15.3	8.6	19.5	7.0	5.3
2	1.4	0.3	3.2	4.1	9.1	1.0	1.1
3	4.6	0.4	4.0	2.9	4.6	1.8	1.9
4	1.5	0.5	3.3	6.0	6.2	0.7	1.1
5	6.6	0.7	0.7	2.8	1.4	1.4	7.4

### III Results from the exercise test 1 year laryngectomy

redicted work load is based upon the age of the subject and unpublished normal material. The maximum work load is the highest sustained for 5 minutes. The values in parentheses refer to the work load at which the exercise test was interrupted, usually after 2 minutes. The  $\dot{V}_E$  and  $\dot{V}_O_2$  were measured at rest in the recumbent position, and immediately before interruption of exercise.

Max work load predicted	(kpm/min) performed	$P_{aO_2}$ (mmHg) At rest/ At work	$P_{aCO_2}$ (mmHg) At rest/ At work
600	200 (400)	65/80	38/36
600	600 (800)	not measured	
600	600 (600)	72/90	40/36
600	1 000 (1 000)	94/91	38/39
900	600 (600) <sup>a</sup>	78/92	41/42

<sup>a</sup> surgical revision of the stoma suby no 5 performed 1 900 kpm min for 6 minutes

Subject 1 fell from 6.2 to 3.6 cm  $H_2O$  (l/s). Subject 2 did not experience any sensation related to this fall of resistance.

Subjects 2-4 had normal or even high working capacity one year after operation according to standards and those of Åstrand (1960).

Subject no 1 had preoperatively a low working capacity which was further reduced postoperatively. Subject no 5 demonstrated a moderate reduction of his working capacity, well correlated with dyspnoea.

This subject suffered from a stenosis of the tracheostoma due to shrinking, which developed 6-12 months after operation.

As a consequence the functional resistance, measured from pleura to atmosphere during the exercise test, was considerably increased and higher than  $R_{th}(1)$  measured from pleura to trachea (Table II).

After surgical revision of the tracheostoma the working capacity became normal (900 kpm/min). The other subjects showed normal values of  $R_{th}(1)$  and  $R_f(1)$ . They had usually small values of tracheal resistance.

At rest  $P_{aO_2}$  was at about the lower limit of the normal range in 2 subjects,  $P_{aCO_2}$  was normal (Table III). During exercise, blood gases

remained at all loads in all patients.

### DISCUSSION

In 5 out of 10 patients the investigations could be completed. These 5 patients were of an age and had a history of smoking corresponding to most subjects with laryngeal cancer.

The pulmonary disease in one patient (no 1) may have influenced the results. The course, however, was similar in the 5 patients. Furthermore, the incomplete data from patients not completing the investigation show the same pattern. In spite of the small number of patients the uniform results make us believe that the results in a representative way show how the lung function is affected by laryngectomy.

Complications such as tracheitis and bronchitis often follow the disconnection of the upper airways from the respiratory pathway (Ingelstedt, 1956; Toremalm 1960). These problems tend to diminish after a varying period of time. Heyden (1950) could not demonstrate any change of lung function or of working capacity 1½-7 months after laryngectomy. Torjussen (1968) reported that disturbed lung function after laryngectomy was often caused by stenosis due to shrinking of the tracheostoma. Fourage (1964) showed that no major deterioration of spirometric data followed 1-2 months after laryngectomy. Most of his patients had low values of forced expiratory and inspiratory volumes, FEV<sub>1</sub> and FIV, respectively, in the preoperative study. In several cases FIV improved considerably due to the elimination of the extrathoracic obstruction caused by the cancer. The values of FEV<sub>1</sub> were often unchanged. In the light of our data this may be due to the development of an obstruction in the intrathoracic airways instead of the eliminated cancer. Fourage (1964) showed that no significant changes of blood gases existed 1-2 months after laryngectomy.

No previous study of pulmonary resistance or elastic properties after laryngectomy seems to have been done. Nor have previous authors repeated the determinations on several occasions postoperatively. The determination of the resistance of the lungs up to trachea has not only the advantage that obstructions due to the cancer

or tracheostoma are excluded. It also increases the sensitivity of the method as concerns its ability to detect obstructions in the lower airways, as the resistance of the extrathoracic airway, already in normal subjects high and variable, is bypassed.  $R_{\text{th}}(1)$  was on average, 0.5 cm  $\text{H}_2\text{O}/(1 \text{ s})$  preoperatively (subject no 2-5). Accordingly, no bronchial obstruction could be shown preoperatively in subjects 2-5, in spite of their heavy smoking habits and as concerns subject no 4 clinical signs of bronchitis. It must be pointed out that the administration of atropine to 4 out of 5 subjects may have eliminated reversible bronchoconstriction.

The poor recoil of the lung preoperatively may to some extent be explained by a higher age in our patients compared with our normal subjects (Fig 1) (Mead et al., 1967). It might also be due to an increased tendency to bronchial closure at low lung volumes, a phenomenon which is a likely explanation of the high RV as well (Jonson, 1970b). The normal values of resistance should not be taken as a proof of healthy airways in our patients in the preoperative study. We can, however, conclude that the obstruction of the extrathoracic airway due to the cancer observed in subjects 1, 3 and 5, had caused no major changes in the intrathoracic airways.

The high values of  $R_{\text{th}}(1)$  obtained postoperatively with the flow regulator method at a standardized value of elastic recoil pressure reflect specifically changes of the bronchial tree (Jonson, 1970a and b, Colebatch et al., 1973). Because of the lengthy procedure of the investigation measurements could be only repeated at a few occasions after bronchodilation. The sparse data show an inconsistent and incomplete effect of adrenalin on the obstruction which indicates that bronchospasm is not of major importance as a causative factor. No patient suffered from crustal tracheo-bronchitis. The nature of the obstruction is not clear. Griffith & Friedberg (1964) and Schwab (1955) have shown that changes in the trachea essentially in the form of metaplasia and chronic inflammation will follow laryngectomy. How far such changes extend into the deeper airways is not known. In

our investigation the obstruction does not seem to be situated in airways smaller than 1 mm in diameter, since airway closure will before higher values of residual volume and a further decrease of elastic recoil pressure could be expected at changes in small airways (Jonson, 1970b).

The most likely explanation for the increased lung resistance postoperatively is an obstruction due to an inflammatory state in the larger bronchi. During the first months after laryngectomy the patients had more or less pronounced symptoms such as tightness of the chest, expectoration and cough. Such symptoms appear in the postoperative material to be very well correlated in time and intensity to the changes of  $R_{\text{th}}(1)$ . The symptoms and signs are well compatible with tracheo-bronchitis. One patient (no 5) experienced increasing dyspnoea during exercise and a reduced working capacity after one year. Our measurements showed that  $R_{\text{th}}(1)$  was normal. The functional resistance, including the resistance of the tracheostoma, was high. After removal of the stoma the patient had a normal working capacity and no dyspnoea.

Reasonable causes for the development of bronchial obstruction are elimination of the conditioning of the inspired air and disturbed mechanisms for elimination of secretions.

All patients were treated with an artificial nose (Toremalm, 1960) after the operation. After 2 to 3 weeks the use of tracheal mask and artificial nose was discontinued. They were then instructed to carry moist gauze compresses over the tracheostoma and drip water into the trachea. All patients have continued to carry dry gauze compresses continuously. Some of them have spontaneously constructed mechanical devices for the gauze, so as to keep it moist in front of the stoma and avoid its interference with inspiration. The patients experienced that those measures and a loose arrangement of clothing around the stoma facilitated the breathing. We feel that there is a need for a specially available device giving effective conditioning permanently.

The elimination of secretion depends

lar function and the mechanism of cough ciliar function is lost to the extent that ous metaplasia occurs (Schwab, 1955, th & Friedberg, 1964) The effectiveness of is considerably decreased as the function tis is lost The patients in time acquire a ique to improve the effect of their cough nspire to a high lung volume and simulate nction of glottis, by occluding the stoma r example, a compress, prior to the cough y they reach the high flow rates necessary pectoration This cough technique should ight early and if possibly trained preopera-

The prominent reduction of R<sub>th</sub>(I) nng during the period 6-12 months after tion shows a remarkable ability of the air to adapt to the poor air conditioning and i mechanism

a pattern of breathing at rest is very variable asily disturbed by emotional factors and ore difficult to study It has been reported review see Fourage, 1964) that the breath tern appears uneconomical in laryngecto- patients during the first few months after tion We have no data which allow us to whether this was the case with our patients s quite obvious, however, that the patients arge problems to control voluntarily the atory manoeuvres during the tests These ems have certainly contributed to changes ed in the static lung volumes especially tal lung capacity 2 weeks to 2 months after tion The unchanged elastic recoil of the after laryngectomy, does not indicate any e of the parenchyma responsible for a de- of TLC Later the TLC returned to values ar to those observed preoperatively

C was first decreased as has also been re- d by Bernhardsgrütter et al (1955) but then ased to preoperative values A reasonable nation for this may be a disturbed nervous ol of the respiration during the first few hs after laryngectomy e breathing pattern during exercise is less ed by emotional factors One year after gectomy the breathing pattern during exer- was similar to that observed in normal sub-

jects, as concerns tidal volumes, frequencies, flow patterns and pleural pressures This means that the patients did not procedure pleural pressures above 2-6 cm H<sub>2</sub>O at heavy exercise, which is about optimal (Olafsson & Hyatt, 1969, Jon- son, 1970, 1971) The nervous control of the ventilatory pattern during exercise was adequate in our subjects one year after laryngectomy

In summary one year after laryngectomy the lung function is in general remarkably good as judged from arterial blood gases, static lung volumes, resistive and elastic properties, nervous control of ventilation and working capacity During the period of adaptation the patients suffer from pulmonary symptoms In subject no 1, who had reduced lung function preoperatively, the symptoms were severe In order to reduce the evident risk for such patients and the troubles for the patients without major lung disease it is desirable to improve pre and postoperative treatment

## ZUSAMMENFASSUNG

Es wurden die mechanischen Eigenschaften der Lunge bei fünf Patienten vor und nach einer Laryngektomie untersucht Die verwendete Methode gewährleistete dass eine Larynxstenose vor oder eine Stenosierung des Tracheostomas nach der Operation die zu messenden Lungenparameter unbeeinflusst liessen Praoperativ wurde zwischen Trachea und Pleura ein normaler pulmonaler Widerstand gemessen Dieser nahm jedoch postoperativ zu um nach etwa sechs Monaten bei ca zehnfacher Steigerung sein Maximum zu erreichen Ein Jahr nach der Laryngektomie war der pulmonale Widerstand wieder auf das Zweifache seines Ausgangswertes zurückgegangen Zu dieser Zeit lag in bezug auf Arbeitskapazität und Blutgase eine normale Lungenfunktion vor Auf diese Weise konnte eine weitgehende Adaptationsfähigkeit der Luftwege an die nach einer Laryngektomie veränderten Ventilationsbedingungen dargestellt werden Gefahren und Probleme während dieser Adaptationsperiode werden diskutiert

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## FUNCTION OF THE LOWER OESOPHAGEAL SPHINCTER IN A POPULATION SELECTED AT RANDOM

*A Manometric, Radiological, and Questionnaire study*

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(Received January 15, 1974)

Using a manometric technique, incompetence of the lower oesophageal sphincter (LES) was found in 14 of 40 persons selected at random. The women predominated with respect to LES incompetence. The mean pressure difference between the LES and the stomach was 10 mmHg in the subjects with LES incompetence and 15 mmHg in the others. Six of those with LES incompetence were found to have hiatal hernia. Dysfunction of the oesophagus was common in the subjects with LES incompetence. On X-ray examination, one of

the subjects gave a history of symptoms suggesting reflux. The symptoms are graded on a scale. The difference between the competent and incompetent LES is statistically highly significant ( $p < 0.001$ ). The symptoms from organs other than the oesophagus and the vagus nerve described by subjects with competent and incompetent LES are compared. The mechanism of the oesophageal sphincter is dis-

examined to demonstrate hiatal hernia and/or gastro-oesophageal reflux have been recommended. Sommer & Stevenson (1961) used an angulated table, the patient lying prone with the epigastrium pressed against the angle of the table. Johnstone (1951) examined his patients in the standing position, bending forwards as if to touch the toes. Turning over from the supine to the prone position is recommended by some as the best manoeuvre to reveal hernia (Flood et al, 1953). Different ways of increasing the intra-abdominal pressure have also been used. Coughing, taking deep breaths, or performing the Valsalva or Mueller manoeuvres have been recommended. Inflating a rubber belt placed round the upper abdomen has been practiced by Marchand (1952) and Sandmark (1963b). Sandmark connected the rubber bag to an electric fan, and with the aid of an autotransformer he was able to maintain a constant pressure at various chosen levels. Wolf (1960) and Stein & Finkelstein (1960) made the examination with the patient lying down and actually swallowing the contrast medium. Others examine the patient after the contrast medium has been swallowed. Most authors use heavy barium contrast media (spec grav 1.4-2.0). Sandmark (1963b) used light contrast medium (spec grav 1.0), with an improvement in diagnostic accuracy.

There are differences concerning the incidence of sliding hernia and reflux in a normal population (Skinner & Booth, 1970; Venkatesan et al, 1972) and in persons with gastrointestinal symptoms (Beck, 1954; Stein & Finkelstein, 1960). This is undoubtedly explained by different methods of examination and different criteria for the diagnosis of hiatal hernia and reflux. Many different methods of X-ray

work was supported by the Swedish Medical Research Council, project No. B74-17X-4260-01 (Tibbling).

When radiology reveals hernia or massive gastro-oesophageal reflux in the recumbent position without extra abdominal compression, the diagnosis may be taken as settled. Many radiological techniques involve some artificial means of uncontrolled increase in the extra abdominal pressure resulting in an unknown increase in the intragastric pressure. Such an increase in the intragastric pressure may in turn give rise to reflux and to hernia in normal subjects. The results of radiology carried out when applying extra abdominal compression to demonstrate gastro-oesophageal reflux and hiatal hernia must be assessed in relation to other non radiographic findings and to the history, if they are to be accepted as reliable in distinguishing between normal and pathological findings.

Symptoms due to dysfunction of the oesophagus have been imperfectly interpreted owing to the lack of a manometric technique in the routine examination of the oesophagus. There can be no doubt that many symptoms in patients with oesophageal dysfunction are wrongly attributed to other neighbouring organs also innervated by the vagus nerve. Bennett & Atkinson (1966) suggested that angina pectoris may often be of oesophageal rather than of cardiac origin. Hiebert & Belsey (1961) and Urschel & Paulson (1967) found an unexpectedly increased incidence of oesophageal dysfunction among sufferers from bronchitis, obstructive bronchitis and laryngitis.

The purpose of the present investigation was to study the incidence of pathological manometric findings in a series of subjects selected at random and to establish the value of X ray examination with controlled abdominal pressure in revealing hernia and reflux. Further aims were to correlate the objective findings to the symptoms and to compare any histories suggesting disorders of neighbouring organs innervated by the vagus nerve in subjects with normal and abnormal LES function.

## MATERIAL

From the population of a Swedish town with 36 028 inhabitants five women and five men

were selected at random from each of the age groups 15, 25, 75. Thus 70 persons were invited to take part in the investigation. 10 declining were asked a second time. For the final selection, 24 men and 16 women. Their age distribution is shown in Fig. 1.

## METHOD

The investigation consisted of manometry, X ray studies and a questionnaire. The results were statistically evaluated by Student's *t*-test.

### Questionnaire

The questionnaire contained seven questions concerning symptoms such as heartburn, regurgitation, lump in the throat, chest pain, heart pain, gall bladder and gastric distress, and weight, number of deliveries, tobacco consumption, dentures and current drug therapy.

### Manometry

The manometric study was performed with a water filled polyethylene catheters of 0.1 mm inner diameter connected to external pressure transducers (sensitivity  $\pm (0-100)$  mm Hg, Elema-Schönander, Sweden). Each catheter had one lateral hole of 2 mm diameter placed 1 cm from the tip. Intra luminal pressures were recorded with three catheters 1 cm apart or in three catheters 5 cm apart. The manometers used were of type 34 (Elema-Schönander, Sweden). The pressure signals were graphed on a multichannel direct recorder (Elema-Schönander, Sweden) with a paper speed of 1 cm/second. The catheters were placed in the distal part of the oesophagus and a stomach electrode (LKB Beckman Instruments AB, Sweden) connected to a Zero reference electrode (LKB Beckman).

The respiration was manometrically recorded from a small air filled balloon attached to the lower chest and connected to a pressure transducer.

The intra luminal tonus pressures were recorded simultaneously at three points 1 cm apart. The tube assembly was withdrawn in 0.1 s.

from the stomach to the pharynx at intervals of at least two breathing cycles

The motility pressures of the oesophagus were recorded simultaneously at three points 5 cm

The catheters were withdrawn in 1 cm at intervals of up to 1 minute depending on the duration of the peristaltic wave. Oesophageal motor function was studied during swallowing. The subject first swallowed water then swallowed dry, or vice versa. The tone and motility determinations were repeated once.

The last feature of the manometric examination: extra abdominal compression was applied by means of a fan attached to a 15 cm-wide rubber belt round the upper abdomen, as described by Sandmark (1963b). The pressure was increased in controlled steps from 0–100 mmHg. The pressure belt was insufflated while the catheter assembly was lying with one hole in the chest and two holes in the oesophagus 5 cm apart, the distal one close to the cardia. When reflux occurred 100 cc of 0.1 N HCl was introduced into the stomach, and the extra abdominal pressure was increased once again as described above.

The subjects were requested not to smoke or eat 4 hours before the manometric and X-ray examinations. The tube assembly and the pH electrode were passed through the nose after nasal anaesthesia, and the subjects were positioned in the supine position.

At each point the pressure at a certain point was registered with three different catheters and the results were repeated once, six values for the intraluminal pressure were obtained from the same point. Each figure given in the results represents the mean of the six recordings.

Examinations were performed on all 16 subjects except 2 (one was pregnant and one did not). Two men who were shown by manometry to have a hernia or an extremely low pressure gradient between the LES and the stomach were also examined. The radiological technique was as follows. The

subjects were examined while swallowing barium suspension (spec. grav. 1.4) in the supine, right anterior oblique position (RAO position) with and without extra abdominal pressure of 100 mmHg applied (Sandmark, 1963a), and in the standing position with no extra abdominal compression. During normal breathing, serial films of the hiatal region were taken at one frame per second with a 70 mm camera. Fluoroscopy was used.

The subjects were also examined after having taken about 100 ml contrast medium in the supine RAO position with the foot end at the table elevated 15 degrees. The subject was asked to breathe deeply and to perform a Valsalva manoeuvre, and the extra-abdominal pressure was raised in steps from 0–100 mmHg. The hiatus region was examined by fluoroscopy and spot films were taken. Finally, with extra abdominal pressure still applied, they were asked to turn from the supine to the prone position.

The diagnosis of hernia was made on the basis of one of the following criteria:

- 1 A complete Schatzki ring (Schatzki & Gary, 1953),
- 2 An incisura cardia above the hiatus,
- 3 Obvious signs of gastric mucosa above the hiatus in combination with widening of the hiatus.

## RESULTS

The following definitions are used and they all refer to the supine position:

- 1 By *reflux* is meant either the decrease of pH or a pressure increase in the distal part of the oesophagus with an extra abdominal pressure of 100 mmHg or less applied.
- 2 *LES incompetence* means reflux as defined above or no pressure gradient between LES and oesophagus.
- 3 *Oesophageal dysmotility* is indicated by,
  - (a) Propulsion of the motility wave on dry swallowing at a rate exceeding 6.6 cm per second in at least two tests,



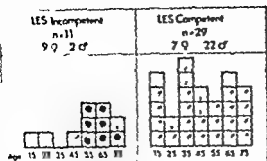


Fig 1 Age and sex distribution in the LES-competent and LES-incompetent respectively ●—Hernia

(b) The following of the peristaltic wave by tertiary contractions on every occasion

#### Manometry

Eleven subjects had LES incompetence, all but one with demonstrable reflux (Fig 1). Six of these subjects were found to have a pressure curve indicating a hernia of less than 2.5 cm (Fig 2). Yet another subject was shown to have a hernia on X-ray examination on the application of an extra abdominal pressure of 100 mmHg.

The subjects are arranged in two groups, LES-incompetent and LES-competent.

In Fig 1 the age and sex distribution of the two groups is shown. The LES-incompetent group includes 9 women and 2 men, and hernias were present in 6 women and 1 man. Since the Competent and Incompetent groups differed with respect to age distribution, all the findings are assessed both in the whole series and in the part of the series related to subjects aged 55 years or more. No sex difference can be found with respect to LES incompetence below 55 years of age. However, from 55 years

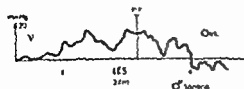


Fig 2 The tonus registration of the intraluminal pressure in one of the LES-competent subjects. The plus and minus refer to the intraluminal pressure change during inspiration. V—ventricle PIP—pressure inversion point Oes—oesophagus

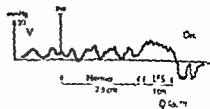


Fig 3 The tonus registration in one of the subjects with a hiatal hernia. For explanation of symbols see Fig 2

and above there is a statistically significant difference ( $p < 0.01$ ) in the incidence of LES competence between the sexes. No hernia found in any subject under 55 years of age.

LES-competent subjects had a mean LES pressure of 7.1 mmHg in the LES at the end of expiration, and in the distal part of the oesophagus of  $\sim 3.4$  mmHg, that is, a pressure gradient of 10.5 mmHg (Fig 4, Table 1). The LES-incompetent group showed a mean LES pressure of 1.8 mmHg and a mean oesophageal pressure of  $\sim 2.4$  mmHg, that is, a pressure gradient of  $\sim 2.4$  mmHg (Fig 5, Table 1). The difference between the groups is highly significant ( $p < 0.001$ ). During inspiration the intraluminal pressure in the oesophagus and the LES changed proportionally and inversely to each other. The mean gastric pressure increase was 10 mmHg, extra abdominal pressure of 100 mmHg (Fig 6). The pressure increase did not differ between the two groups.

The mean length of the LES calculated from the pressure inversion point to the beginning of the part of the oesophagus showing subsphincteric pressure, and the mean length of the upper oesophageal high pressure zone, was slightly less in the LES-incompetent group than in the LES-competent group. The difference was not statistically significant.

#### Questionnaire

The questionnaire concerning symptoms was ranked on a scale, each symptom being scored according to the frequency with which it occurred. All but one in the LES-incompetent group required more than 5 points (mean 8.5). The competent subjects attained a mean score of

is significantly lower than the other group (0.001, Table I). When comparing the symptoms in the two groups of subjects, aged 55 years or over, the difference was still statistically significant ( $p < 0.01$ ).

Table II shows the incidence of history of heart pain, gall bladder and gastric disorder, and present smoking and dysmotility of

# **Table I The intraluminal pressure of the oesophageal sphincters, ventricle and oesophagus, hi excess, length of the oesophageal sphincter and history score in LES competent and incompetent subjects**

Results are given with standard error of the mean. Asterisks refer to significance limits of  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.001$  (\*\*\*). The values within parentheses are those from the age groups 55 years or

	LES competent (n 29)	LES incompetent (n 11)
ure gradient Hg LES- phagus end of	10.9 ± 1.15 (9.5 ± 1.82)	4.2 ± 0.83*** (3.6 ± 1.07)**
ure gradient ventricle of exp	7.07 ± 1.15 (6.2 ± 1.53)	1.8 ± 0.64*** (2.3 ± 0.70)
ure gradient Hg LES- ricle end of	13.1 ± 0.89 (13.8 ± 1.21)	-10.0 ± 2.55 (11.1 ± 3.34)
ure gradient Hg oeso- phagus-ventricle of exp	-3.4 ± 0.64 (-3.4 ± 1.01)	2.4 ± 0.84 (-1.4 ± 0.66)
ure gradient Hg oeso- phagus-ventricle of insp	10.9 ± 1.38 (12.4 ± 0.96)	-9.8 ± 1.09 (-8.8 ± 1.18)
ure intra-gastric pressure (mmHg)	3.1 ± 0.47 (3.3 ± 0.54)	3.3 ± 0.66 (3.9 ± 0.77)
cially increased gastric pressure Hg	9.3 ± 1.03 (7.7 ± 0.92)	10.7 ± 2.36 (9.4 ± 2.23)
h of LES (cm)	3.5 ± 0.27 (3.1 ± 0.23)	2.8 ± 0.35 (2.5 ± 0.37)
h of upper phagus high sure zone (cm)	3.52 ± 0.27 (3.09 ± 0.23)	2.8 ± 0.35 (2.5 ± 0.37)
hi excess (%)	7.6 ± 2.87 (7.5 ± 2.91)	10.9 ± 3.48 (13.5 ± 4.3)
tionnaire score	2.6 ± 0.55 (3.3 ± 0.99)	8.5 ± 0.90*** (8.9 ± 1.20)**

## **Table II History of heart pain, gall bladder and gastric disorders, smoking habits, full dentures, and occurrence of oesophagus dysmotility in subjects with competent and incompetent LES**

Values in parentheses refer to subjects 55 years and over. For explanation of asterisks see Table I.

	LES incompetent	LES competent
Heart pain	7/11 64% (6/8 - 75%)*	4/29 - 14% (3/12 - 25%)*
Gall bladder	2/11 18% (2/8 - 25%)*	5/29 17% (3/12 - 25%)*
Gastric disorder	3/11 27% (3/8 - 38%)*	5/29 17% (1/12 - 8%)*
Smoker	4/11 36% (1/8 - 13%)*	10/29 35% (0/8)
Ex smoker	1/11 9%*	8/29 28%*
Full dentures	4/11 36% (4/8 - 50%)*	4/29 14% (2/12 - 17%)*
Dysmotility of oesophagus	6/11 - 55% (5/8 - 63%)*	2/29 = 7% (0/12) **

the oesophagus. In evaluating the variations in the different parameters it must be taken into consideration that most of them are age dependent, several are sex dependent, and some are interdependent. Since there are very few subjects below 50 years of age in the LES incompetent group, statistical assessment is only carried out on the subjects 55 years of age or over. The

## **Table III Grading of symptoms in gastro-oesophageal reflux**

The symptoms are ranked on a scale from 4 to 1. Only one of the symptoms under 4 points is to be included in the sum of points for each individual.

### **4 Points**

Heartburn at rest or heartburn on bending forward or heartburn on exertion or regurgitation.

### **2 Points**

Lump in the throat, epigastric tenderness, excess of pharyngeal mucus, history of heartburn at end of pregnancy, chest pain on exertion after meals.

### **1 Point**

Substernal pain provoked by exposure to cold, substernal pain provoked by emotional stress, other attacks of substernal pain, attacks of hiccup, attacks of air regurgitation, cough.

history of heart pain and the incidence of dysmotility of the oesophagus were far commoner among LES incompetent subjects than in those with LES competence.

The smoking habits did not vary between the groups. Full dentures were more often found in the group with LES incompetence. All with full dentures showed either reflux or a disturbance of oesophageal motility or a combination of both.

#### X ray

On X-ray examination four hernias were found. Three of these were also demonstrated by manometry, and the fourth was diagnosed in a woman in whom manometry showed no pressure gradient between LES and the oesophagus.

In 4 other subjects small hernias could not be excluded, but the findings did not quite fulfil the criteria as defined above. Of these 4, one had a hernia, one showed reflux, and 2 showed normal findings in the manometric study. Five of the subjects with LES incompetence had entirely normal X-ray findings. The hernias were demonstrated with the subjects lying down and during the swallowing of contrast medium with maximum extra abdominal pressure applied. One hernia was also filled retrogradely when an external pressure of 60 mmHg was applied. This subject had the largest hernia (6 × 6 cm), and was the only one in whom radiology disclosed reflux. Valsalva manoeuvres did not elicit hernia in any case.

### DISCUSSION

It is probably more important to establish the diagnosis of pathological gastro-oesophageal reflux than a small sliding hiatal hernia. The symptoms of a hiatal hernia are caused by regurgitation (Allison, 1951) and small hernias give regurgitation more often than larger ones (Sandmark, 1963a). Regurgitation and oesophagitis may also occur in the absence of clinical signs of hiatal hernia (Hiebert & Belsey, 1961). Furthermore, hernias shorter than 2 cm are difficult to demonstrate whether by X-ray examination or manometry (Cohen, 1971). In the present investigation radiography disclosed reflux in

only one of 11 patients shown to have reflux by manometry. This tallies with the experience of others (Hiebert & Belsey, 1961; Skinner 1964). Since the history corresponded to the finding of LES incompetence on manometric investigation, this technique for evaluating the function of the LES seems to be advantageous. With 46% (16/35) of the women and 69% (7/10) of the men invited agreeing to take part, one wonders how many of the objectors also had oesophageal dysfunction. Even supposing that all the objectors had a perfectly normal LES, there would still be a female preponderance in respect to LES incompetence (26 ± 10% compared with 6 ± 3.7% in the male subjects).

All pathological radiological findings were in accordance with the history and results of manometry. This is probably explained by the criteria used for diagnosing hernia. If the signs to differentiate a small hernia from a phrenic ampulla had been used, more hernias might have been correctly diagnosed. There probably also have led to false positive results, however, since the signs of gastric folds at the hiatus and obliteration of the incisura angularis and differences in motility between the phrenic ampulla and a herniated part of the ventricle are sometimes difficult to identify. The difficulty of distinguishing normal from pathological border line cases are illustrated by the 4 subjects in whom a small hernia could not be excluded. Of these two were pathological and two were normal on manometry. It is also of interest to note that only one of the diagnosed hernias became retrogradely filled with contrast medium. This may be explained by Sandmark's observation that heavy contrast medium might draw the hernicle into the abdomen and reduce a small hernia (Sandmark, 1963a). The reason why nevertheless succeeded in demonstrating small hernias when the patients were actually swallowing could be that this part of the examination was performed during the first swallow, before any appreciable amount of contrast medium had entered the ventricle.

Since only one of the 11 subjects with LES incompetence was shown to have reflux at rest

hy, and since 5 of them had entirely normal X-ray findings, the X-ray technique seems to be inferior to manometry in demonstrating LES incompetence.

Bennett & Atkinson (1966) claimed that the hiatal hernia was more often due to a distension of the oesophagus than of the heart. Koch (1964) observed a striking relationship between gastric, oesophageal, and heart disorders. Our results show a more frequent history of hiatal hernia in the subjects with LES incompetence than in the others. Concomitant use of other organs innervated by the vagus nerve may be explained in several ways. It may be due to misinterpretation of the symptoms, to affections of the heart and the oesophagus, or to simple overlap. The latter is probably the simplest, as the symptoms are readily confused. Another reason may be a common neurological factor. It has been shown that smoking briefly lowers LES pressure (Dennish & Castell, 1971; Chu & Bennett, 1972). In this investigation subjects were asked to refrain from smoking prior to the investigation. No difference in smoking habits was seen between the 'reflux' and the 'normal' group.

The function of the LES is regulated by several factors, of 'humoral and physical' character (Sjodahl, 1972). Our findings regarding the pressure variations in the cardia and the oesophagus form a basis for a discussion of the possible sphincteric function of the LES. No morphological proof has been forthcoming that the LES is a sphincter. It may be regarded as such on functional grounds, however,

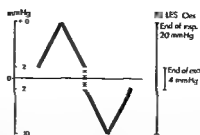


Fig. 5 The pressure gradients between the LES and the oesophagus in the LES incompetent subjects ( $n=11$ ). The figures represent mean values. The zero pressure point refers to the intragastric pressure at the end of expiration.

as the intraluminal pressure in the cardia can be influenced by various drugs and hormones (Castell & Laurant, 1970), like all tubular organs equipped with smooth muscle. Presumably these smooth muscle cells in the submucosa are necessary to close the lumen of the LES. Beyond this closing mechanism, a valve mechanism based on the transdiaphragmatic pressure gradient augmenting the competence of the LES seems reasonable on the following ground. During inspiration the fall in the intra-oesophageal pressure is accompanied by a rise in the intracardial pressure (Figs 4 and 5). The increasing pressure gradient during inspiration will cause a dynamic compression of the closed tube in analogy with the findings in the trachea of children suffering from an acute attack of croup (Ingelstedt et al., 1967). The valve mechanism which increases the competence of the LES during inspiration is easily seen on X-ray examination of the oesophagus, and has been discussed by Creamer et al. (1959). In order to overcome the dynamic compression of the cardia and to force it to open it is necessary to apply positive pressure exceeding the pressure gradient between the cardia and the oesophagus. In order to cause reflux from the ventricle to the oesophagus, the gastric contents must be forced through the cardia. In our series the mean intragastric pressure of 10 mmHg, lagging behind the extra-abdominally applied pressure by one tenth, seems to have been operative in distinguishing between a competent and an incompetent LES, since the pressure gradient between cardia and

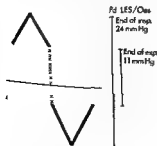


Fig. 6 The pressure gradients between the LES and the oesophagus in the LES-competent subjects ( $n=29$ ). The figures represent mean values. The zero pressure point refers to the intragastric pressure at the end of expiration.

oesophagus in symptomless subjects, in contrast to those with symptoms, exceeds 10 mmHg at end of the expiration (Table I)

## CONCLUSIONS

- 1 In a population selected at random women aged 55 years and over strongly predominate with respect to LES incompetence
- 2 The methods of X-ray examination used in this investigation are not reliable for demonstrating gastro-oesophageal reflux
- 3 The symptoms can be graded and the total score used in screening for oesophageal dysfunction
- 4 The competence of the LES is partly due to the dynamic compression obtained by the transdiaphragmatic pressure gradient
- 5 Gastro-oesophageal reflux takes place when the intragastric pressure exceeds the pressure differences between the LES and the oesophagus

## ZUSAMMENFASSUNG

Mittels einer manometrischen Methode wurde bei einer wahllosen Auswahl von vierzig Personen in elf Fällen — überwiegend bei Frauen — eine Inkompetenz des unteren Ösophagusphinkters (LES) gefunden. Die durchschnittliche Druckdifferenz zwischen LES und dem distalen Teil des Ösophagus bei Expirationsende betrug 4,2 mmHg bei den LES inkompetenten Patienten und 10,5 mmHg bei den übrigen Sechzehn von den LES inkompetenten Patienten hatten einen Hernie-Hiatus Dysmotorik des Ösophagus überwog bei den LES inkompetenten Patienten. Bei röntgenologischer Untersuchung fand man bei einem der elf LES inkompetenten Patienten einen gastroösophagischen Reflux und bei vier Patienten einen Hernie-Hiatus. Alle der LES inkompetenten Patienten ausser einem hatten eine Anamnese, die auf einen Reflux hindeutete.

Die Symptome wurden nach einem Punktsystem graduiert. Die Differenz der Punktsumme zwischen den LES kompetenten und -inkompetenten Patienten ist statistisch dreisternig signifikant ( $p < 0.001$ ). Die Symptome bei anderen Organen, die vagusinnerviert sind, wurden auch zwischen den LES kompetenten und inkompetenten Patienten verglichen.

Diskutiert wurde auch der Ventilmechanismus des Ösophagus.

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## IN VITRO ANTIBODY PRODUCTION OF HUMAN TONSIL FOR SHEEP RED CELLS (SRC)

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(Received November 17 1973)

**Abstract** An attempt to determine the capacity of lymphocytic suspension from human tonsils to produce antibodies in vitro against sheep red cell was conducted using Marbrook's culture technique. Detection of the antibodies was made using Cunningham's plaque forming method. It was confirmed that the plaque forming cells increased predominantly on the 6th day of culture. There was no relationship between the age of the patient and the plaque forming capacity. The immunological roles of the human tonsil with additional reference to E and EAC binding lymphocytes previously reported become more pronounced.

Recent advances in immunological techniques have suggested that human tonsils, as well as regional lymph nodes play an immunological role.

In 1973, Tabata et al reported that subpopulations of lymphocytes from human tonsils were capable of rosette formation with both sheep red cells (SRC) and SRC sensitized with anti SRC antibody-complement. It was then postulated that the human tonsil may well supply two different kinds of lymphocytes possessing immunological properties, such as do the thymus and bone marrow derived cells in mice (Bianco et al, 1970, Lay et al, 1971). In humans, therefore, the tonsils may perform a similar function to that of lymph nodes and produce in vitro antibodies for certain antigens.

The present experiment is an attempt to determine the immunological function of the human tonsil by evaluating the capacity of tonsil lymphocytic suspension to form antibodies against sheep red cells.

### MATERIAL AND METHODS

Experimental materials were obtained from patients with a preoperative diagnosis of tonsillitis. Tonsils due to recurrence of inflammation. There were 3 females aged 9 years and 6 males aged 5 to 11 a total of 9 patients with an age range of 5 to 11 (Table I). The patients were otherwise free of systemic disease or complicating factors. The many advanced immunological techniques available for in vitro antibody production by the plaque forming technique using SRC as antigen was selected for use in this experiment.

#### *Preparation of lymphocytic suspension from human tonsils*

Cell suspension was prepared from surgically removed palatine tonsils and was gently placed on the surface of 15 ml of 6% Amies' solution. Ficoll. The cell suspension was then centrifuged at 1 500 rpm for 10 min to separate the lymphocytes. A confirmation of 90% lymphocyte content in the lymphocytic layer remaining in the test tube was made.

#### *Preparation for culture*

Marbrook's technique for in vitro culture was slightly modified (Fig 1) and used as a standard procedure. RPMI 1640 synthetic medium supplemented with 10% of fetal calf serum, 100 U/ml streptomycin, and 100 unit/ml penicillin.

# Clinical description of patients

Patient	Sex	Age
K M	♀	7
T Y	♀	9
T T	♀	6
K F	♀	7
T I	♀	6
T S	♀	5
T F	♂	11
T K	♂	8
T K	♂	11

ad was buffered with sodium bicarbonate  $2 \times 10^7$  cell suspension was placed into the r culture compartment containing 10 ml lture medium, and SRC were then added mixed. The lower compartment with 9 ml lture medium was effectively shielded with lysis membrane. A control culture without en was prepared and placed in the upper eriment. The culture compartments were d in a humidified incubator at  $37^\circ\text{C}$  in an sphere of 5%  $\text{CO}_2$  in air

en  
 preserved in Alsever's solution were oughly washed with Hanks balanced salt ion before use. The optimum dose of SRC ers was determined

## Detection of antibody production by plaque-forming cells (PFC)

The plaque forming capacities of the cell suspension of the in vitro culture with SRC were detected according to Cunningham's direct method of hemolytic plaque assay for 19S antibody-producing cells (Fig 2). An outline of this procedure is summarized in Fig 3.

## Cell viability

Trypan blue staining (0.5%) was used to detect the viability of the culture cells. The percentage of survival cells per 200 cells was then determined.

## RESULTS

Cell viabilities were observed in the cultures for a period of 10 days (Fig 4). There was a 40–50% survival rate of cells throughout the culture period. An apparent decrease of viability occurred on the second day.

Optimum dose of SRC antigen was determined on the 4th day of cultures of the tonsil cell suspension in good condition. Table II demonstrates the plaque forming capacity of  $2 \times 10^7$  tonsil lymphocytes with in vitro stimulation due to different concentrations of SRC on the 4th day of culture. Five cases showed an increase of the plaque forming cells (PFC), in a case of  $10^6$  of SRC added. A maximum number of PFC was 557. Control culture without

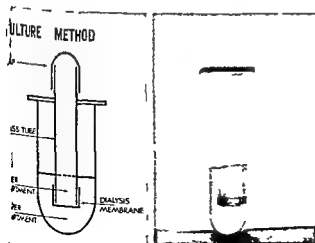


Fig 1 Schematic drawing of culture apparatus and the photograph



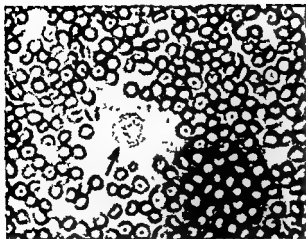


Fig 2 An arrow indicates plaque forming cell Phase contrast  $10\times 40$

antigen, however, showed a marked lower capacity to form plaques. From this preliminary experiment, it was confirmed that optimum dose of SRC was  $10^4$  cells.

Table III demonstrates the time course of plaque forming capacity in 4 cases. The tonsil cell suspensions of  $2\times 10^7$  were cultured with  $10^4$  of SRC. There was a prominent increase in plaques from Day 4–6 of culture in all specimens, although with individual differences. The highest value was found on Day 6 of culture in the case of a five year old male, with a reading of  $1332\pm 169$ . No correlation between patient age and capacity to form plaques was observed.

## DISCUSSION

In 1963, Ambrose successfully devised a method of culturing rabbit lymph nodes for the *in vitro* production of antibodies. An improved culture technique was also reported by Ortiz-Muniz & Singel (1967). Surjan & Surjan (1968–1970) attempted an *in vitro* study of antibody production in rabbit tonsils and postulated that rabbit tonsil may take part in the immunological process. Tabata et al (1973) reported no *in vitro* response of rabbit tonsil to antigens such as diphtheria and bovine serum, although peripheral lymph nodes produced antibodies, and concluded that rabbit tonsil plays a role of anti-

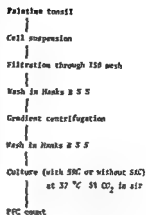


Fig 3 An outline of general procedure

gen messenger but not of antibody production. Apart from *in vitro* study of rabbit tonsils, investigations on the immunological function of the human tonsils exist because of the studies of immunological and *in vitro* techniques.

Surján et al (1971) reported that the human tonsils immunized against diphtheria, pertussis and tetanus toxoids were able to produce antibody by tissue culture technique using appropriate unit of phytohemagglutinin (PHA) stimulants. The use of PHA, however, all antibody titer as determined by the hemagglutination test. Our previous experiments revealed that PHA, even in low concentration,

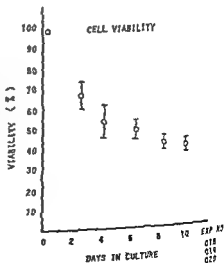


Fig 4 Graphical representation of cell viability throughout culture days

## II The determination of optimum dose of antigen responsible with $2 \times 10^7$ cell suspension of human tonsils

indicate the plaque-forming cells

No. of specimens				
011	012	013	014	106
23	5	157	66	88
—	—	122	80	60
91	73	360	122	165
111	271	557	124	201
62	237	389	90	73
27	—	193	32	55

ed non specific hemagglutination of sheep  
is, therefore, the use of PHA should be

For this type of study, more advanced  
immunological and culture techniques will be  
used in order to obtain the convincing results

present study is an attempt of in vitro  
production of human tonsil using the

techniques such as Marbrook's culture  
Cunningham's hemolytic plaque assay The

plaque technique for detecting anti-  
synthesis at the cellular level described by  
& Nordin in 1963 was recently improved  
Cunningham & Szenberg (1968)

Cunningham's improved plaque technique may  
be of value in future experiments Using

Cunningham's culture technique for lymphocyte  
culture, a 40–50% cell viability was

used SRC was selected as the antigen  
in plaque assay To insure results,

the concentration of SRC was checked

## III Plaque-forming cells per $10^3$ according to culture in cell suspension of human tonsils with added SRC antigen $10^6$

No. of specimens				
018	019	020	021	
41 ± 11	10 ± 5	8 ± 4	11 ± 4	
42 ± 18	14 ± 7	26 ± 5	13 ± 3	
362 ± 97	209 ± 40	204 ± 56	159 ± 16	
1332 ± 169	249 ± 54	193 ± 39	314 ± 120	
690 ± 243	278 ± 128	142 ± 20	119 ± 60	
613 ± 362	78 ± 47	117 ± 26	78 ± 21	

It was determined that  $10^6$  SRC was an optimum dose to  $2 \times 10^7$  tonsil cells

The occurrence of plaque forming cells observed in control cultures without antigen is thought to be related to a non specific reaction between tonsil cell suspension and SRC A similar finding was found in the original report by Marbrook (1967), although the mechanism of non specific reaction is still unknown In the cell culture with SRC antigen, the most predominant plaque-forming capacity occurred around 6th day and continued through the experimental period The result concurs with a usual pattern of antibody production Antibody production against SRC appears to have no relationship to age of subjects used in the experiment Although the present experiment demonstrated the capacity of human tonsil lymphocytes to produce antibodies against sheep red cells, response to other bacterial toxins and allergens remains to be determined If suitable culture conditions can be established, further studies on these factors will be attempted Another interesting point for future study would be an evaluation of cell-coordination in the production of antibodies in the human tonsil

## SUMMARY

In vitro study for antibody production in human tonsils was performed by using the plaque-forming technique Results showed that human tonsil lymphocytes produce specific antibodies to sheep red cell antigen

## ACKNOWLEDGEMENT

We thank D. Greer R.N. for review of this manuscript

## ZUSAMMENFASSUNG

Ein Versuch die Fähigkeit der Lymphozytensuspension der menschlichen Tonsillen zu bestimmen, die den Antikörper in vitro gegen die Schaf Erythrozyte produziert, wurde mit der Marbrook-Kulturtechnik gemacht. Der Antikörper wurde bei der Cunningham's plattenbildenden Methode entdeckt Es wurde bestätigt, dass die plattenbildenden Zellen unter den besten Bedingungen am 6. Tag der Kultur erheblich zunahm. Die Antikörper-

Produktionskapazität war von dem Alter des Patienten unabhängig. Die immunologische Rolle der menschlichen Tonsillen, denen die oben gesagte E- und EAC bindenden Lymphozyten hinzugefügt wurden, hat sich überraschend offenbart.

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## LONG-TERM MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL EFFECTS OF SMALL MECHANICAL LESIONS IN THE GUINEA PIG COCHLEA

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**Abstract** This paper reports the results on chronic morphological and electrophysiological effects of small lesions in 6 guinea pigs. The lesions were made on the external wall on one of the three scalae of the basal cochlear turn. Following a 6 week recovery period the round window was exposed using a postauricular approach and a platinum iridium ball electrode was placed on the round window. Measurements of the CM were made at ten frequencies. Following completion of the electrophysiological measurements, the animals were perfused with Prussian blue solution and their temporal bones removed, fixed and stained, decalcified and prepared for microscopy. In general electrophysiological responses of the cochlea were depressed following discrete mechanical lesioning. Morphologically there was little influence segmentally limited on the vasculature at the site of the lesion. Little histopathological effect was seen on the organ of Corti. Although no consistent relationship was found between individual mechanical lesions primarily restricted to one scala and subsequent electrophysiological effects, findings of this study do indicate that restricted mechanical damage to the cochlea results in restricted histopathology and a restricted effect on cochlear function.

In a previous paper (Hallen et al, 1974), we described the immediate effects of small mechanical lesions made in the external wall of the guinea pig cochlea. The effects of these lesions were studied with both electrophysiological and morphological techniques. Although we did not observe any quantifiable and orderly correlation between size and location of lesion and change

in cochlear activity, it was noted that restricted lesions resulted in restricted changes of both electrophysiology and morphology of the cochlea. The trauma caused by the mechanical lesion was limited to a narrow segment of the external wall with major changes restricted to the surrounding vessels. No histopathological changes were seen in the organ of Corti.

The authors concluded that the combined use of both electrophysiological and morphological techniques provides an effective and appropriate approach for the evaluation of the effects of surgically induced cochlear lesions. We feel that such a combined approach provides distinct advantages over an approach based upon either of those measures alone. The purpose of the present investigation is to extend these observations on the effect of inner ear surgery and trauma. Specifically, the long-term effects of such mechanical lesions on the cochlear microphonic (CM) activity and histopathology of the cochlea are presented.

### METHODS

#### *Subjects*

A total of thirteen young adult guinea pigs weighing between 250 to 400 grams were used in this study. Additionally, 18 guinea pigs were used as a control group of animals in this study for establishing normal CM activity. No lesions were made in these animals. All animals appeared healthy and exhibited a normal

This research was supported in part by NIH grant 508181 and Office of Naval Research (Contract 00014-67 A-0103-0031) the Swedish Board for Technical Development (project Fr 1500 u 1170) Götaverken Pyrad Company and Swedish Medical Research Council (no B74-12X 538-10A).

**Preyer's reflex** After initial observation of middle ear infection in our first preparations, the animals were placed on ampicillin prophylactically for 2 weeks. Electrophysiological and morphological information was obtained on 6 animals. Morphological information only was obtained on 7 animals.

### *Surgical procedure*

**Chronic lesions** Each animal was anesthetized with sodium pentobarbital (35 mg/kg) and placed in a nontraumatic head holder. A small incision was made in the neck which ran inferiorly 1.5 cm lateral to the thyroid gland. The tissue and the trachea were gently retracted and the auditory bulla was identified. The bulla was opened exposing the middle ear space and the cochlea. Lesions were restricted to the basal turn and located about one-fourth of a turn from the basal end halfway between the round window and the bulla wall. The bony wall of the cochlea was thinned with a small diamond drill. A delicate pick was then used to remove the remaining bone. Surgical trauma of the periotic and membranous labyrinth and intracochlear vessels was accomplished with a microdissecting knife in all animals except one, in which a cauterized lesion was made using an insulated electrode with a 5  $\mu$ m diameter uninsulated tip. The lesions were left without sealing the cochlear wall and the wound was sutured.

**Electrophysiology** All electrophysiological recordings of the CM were made from the round window following a 6-week recovery period. The animals were anesthetized with 0.2% Penthrane and placed in a head-holder. The external ear was removed and the auditory bulla was exposed using a post-auricular approach. The bulla was opened, exposing the middle ear and bony capsule. A platinum-iridium ball electrode was placed on the round window, secured with dental cement and the bulla was sealed.

### *Electrophysiological procedures*

Electrophysiological procedures used in this paper have been described elsewhere (Hallen

et al, 1974, McPherson & Miller, 1974). One-half inch condenser microphone (Grain Processing Corporation) and ear-speaker (PDR 600) were coupled to the external auditory meatus. The probe tube was advanced until it was 2 mm lateral to the tympanic membrane. The sound source and probe tube were sealed such that the system presented in a closed system.

Measurements of the CM were made at frequencies of 0.08, 0.20, 0.50, 1.0, 2.0, 6.0, 10.0, 20.0, and 30.0 kHz. In addition to the 1  $\mu$ V isopotential contour, the input (dynamic range) functions of the CM were examined for a variety of frequencies. A Radio wave analyzer was used to record the output of the CM.

### *Histological procedures*

Following completion of electrophysiological measurements, the animals were perfused with Prussian blue solution as previously described (Axelsson, 1968, 1972; Hallen et al, 1974). The temporal bones were then removed and the cochleae fixed and stained with 0.5% osmium tetroxide buffered with Millonig. The bones were decalcified in 10% EDTA buffered to pH 7.0. The cochleae were then washed, dehydrated, and transferred to glycerin. The temporal bones were dissected with the aid of a dissecting microscope. Sectioning of the cochlea was done so that portions of the external spiral lamina, and basilar membrane were mounted on slides and the vasculature of the neuroepithelium, and supporting structures were examined under light and phase microscopy. Details of this preparation have been described elsewhere (Axelsson et al, 1974).

Of the 13 animals subjected to surgery, 6 showed secondary complete fibrosis of the cochlea, apparently resulting from labyrinthitis. One animal had a circumscribed labyrinthitis in the basal part of the scala tympani. The site of the lesion. Only those 6 animals with both electrophysiological and morphological findings were achieved are presented here. The morphological findings in the additional 7 animals were in agreement with those pre-



**Fig 1** Animal 1 (318) Modiolus and most apical part of a vestibuli, basal turn, apico-basal section. In a part corresponding to the lesion (limits  $\perp$  -  $\perp$ ), there are four radiating arterioles of which one is completely injected (small arrow), two are lightly injected (the other ones) and one is well injected. Other radiating arterioles (RAL) are well injected. There is a gap in the "glomeruli" of the modiolus (large arrow) corresponding to the lesion. There is a delicate collecting duct running spirally in the most apical part of the vestibuli.

by abnormal findings are reported. Omission of comment indicates that electrophysiological and morphological features were characteristic of the normal guinea pig.

#### Animal No 1 (318)

**Perforation** An elongated fistula was made apically in the scala vestibuli just beneath the second and three radiating arterioles were divided. There was some leakage of perilymph.

**Morphology** The hole in the bulla was almost sealed with the remaining hole sealed by a thin membrane. There was a bony ridge from the



SVS

**Fig 2** Animal 1 (318) Basal part of scala vestibuli and a part of scala media basal to the lesion, apico-basal section. There is a moderate gap in the vasculature corresponding to the lesion with two uninjected radiating arterioles (arrows). This part of the spiral ligament and vasculature (SVS) is less well stained.



**Fig 3** Animal 1 (318) Scala media, basal turn, apico-basal section. Basally to the lesion there is a part of the external wall which is less well stained and where some of the capillary loops in the stria vascularis are less well contrast injected.

bulla wall over to the basal turn. Pathological peroneum vessels were noted in this region. A small bony protuberance was noted at the site of lesion. The lesion appeared to have affected at least four radiating arterioles of which one was seen to be well injected and three were less injected with a gap in the "glomeruli" and in the apical part of the scala vestibuli in the area corresponding to the lesion and also basally to the lesion (Fig 1). Some capillaries in the basal part of the scala vestibuli (Fig 2) and in the apical part of the stria vascularis corresponding to the lesion were uninjected (Fig 3). The vessel of the spiral prominence was comparatively poorly injected in this region. In the region corresponding to the lesion, the attachment of Reissner's membrane in the external wall was less prominent than in other regions and the membrane was folded there. There appeared to be only minor changes in the organ of Corti with some indication of scattered early degeneration of outer hair cells restricted to the region of the lesion.

**Electrophysiology** There was a slight increase in sensitivity of the  $1 \mu V$  isopotential curve for low frequencies (Fig 4). The dynamic range for 2 000 (Fig 5) and 4 000 Hz (Fig 6) showed a slight change in the slope of the dynamic range, however, the maximum amplitude was within the range of the control animals. The input-output function at 10 000 Hz (Fig 7) was within the normal range.

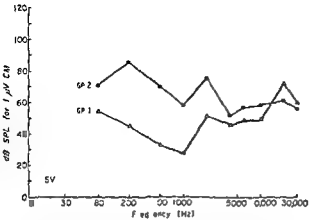


Fig. 4. Animals 1, 2 (318-343). Change in the 1 µV sensitivity contour, following lesion restricted to scala vestibuli. The shaded area shows 2 SD about the mean value for a control group of animals.

Animal No. 2 (343)

**Surgery:** The wall of the scala vestibuli was thinned and a small fistula made immediately below the second turn. There was some leakage of perilymph. Two radiating arterioles were divided mechanically.

**Morphology:** There was a large bony overgrowth at the site of the lesion in the basal turn with some apparent pathological vessels on the outside of the cochlea. There was also a 'arrow gap' in the radiating arterioles in the

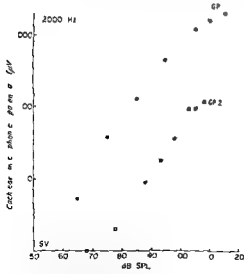


Fig. 5. Animals 1, 2 (318-343). Input-output function of the cochlear microphonic at 2000 Hz for lesion made in scala vestibuli. The shaded area represents 2 SD about the mean value for a control group of animals.

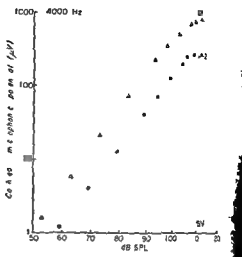


Fig. 6. Animals 1, 2 (318-343). Input-output function of the cochlear microphonic at 10000 Hz for lesion in scala vestibuli. The shaded area represents 2 SD about the mean value for a control group of animals.

most apical part of the scala vestibuli probably affecting two radiating arterioles. The branch in the basal part of the scala vestibuli was, however, contrast injected. Two or three radiating arterioles lack the normal smooth appearance of the vessel wall (Fig. 8). There was a decrease of pigmentation in the vasculature corresponding to the lesion and capillaries were less well injected here at the attachment of the vestibular membrane.

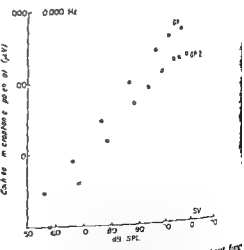
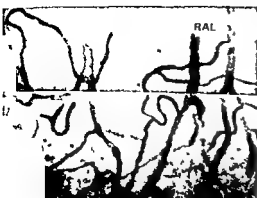


Fig. 7. Animals 1, 2 (318-343). Input-output function of the cochlear microphonic at 10000 Hz for lesion in scala vestibuli. The shaded area represents 2 SD about the mean value for a control group of animals.



SVS

Fig. 9 Animal 2 (343) External wall scala vestibuli and scala media apico-basal section. Basally to the lesion the scala vestibuli the radiating arterioles (RAL) and capillary branches in the basal part of the scala vestibuli are contrast injected (right half of picture). The vessel wall, however, seems more uneven than normal and the capillaries somewhat dilated. The stria vascularis (SVS) are less well defined.



Fig. 10 Animal 3 (344) External wall scala vestibuli and scala media apico-basal section. Apical to the lesion (horizontal arrow) four radiating arterioles (RAL) show empty vessel lumen (see Fig. 10). To the left of the lesion, parts of the vestibular membrane are folded back (vertical arrow). There is a thinning of the spiral ligament basally to the lesion particularly prominent in the stria vascularis (SVS) (see Fig. 11).



Fig. 10 Animal 3 (344) Scala vestibuli apical to the lesion apico-basal section. Four of the radiating arterioles (RAL) show empty vessel lumen (arrows). Vessel walls are evident on each side of each arrow.

more prominent and uneven than usual and there was an increased amount of pigment in the membrane corresponding to the lesion. On the whole, there seemed to be little influence on the vascular pattern of the external wall. Degeneration of outer hair cell stereocilia was observed restricted to the lesion site that did not appear due to preparation artifact.

**Electrophysiology** There was a decrease in the sensitivity of the 1  $\mu$ V isopotential curve for this animal (Fig. 4). This was true for both low and high frequency with maximum change occurring at 2000 Hz (25 dB)<sup>1</sup>. The average decrease in sensitivity was approximately 20 dB. The high frequencies 20 000 and 30 000 Hz were within normal limits as well as the lower limit of 80 Hz. The dynamic range at 2000 Hz (Fig. 5) showed a marked reduction as well as a reduction in the dynamic range at 4000 and 10 000 Hz (Figs. 6 and 7).

#### Animal No. 3 (344)

**Surgery** A small fistula was made in the scala vestibuli just at the junction to the second turn. A mechanical lesion was made seemingly limited to one single radiating arteriole.

**Morphology** The endosteum vessels in the basal turn of the cochlea were uninjected corresponding to the point of lesion. There was a bony ridge going over from the bulla wall to the scala vestibuli and scala media in the basal

<sup>1</sup> Decibel change is computed from the mean value of the control animals.





Fig 11 Animal 3 (344) Scala media basally to the lesion on apico-basal section. There is a narrow segment in the stria vascularis (SVS) with pigment deficiency and empty capillaries

turn and the lesion was completely healed with bone. There was a gap in the vasculature of the external wall apparently emanating from four or five radiating arterioles (Figs 9 and 10). At the level of the lesion there was narrow avascular gap in the stria vascularis and a narrow segment of deficient pigmentation (Fig 11). Arterio-venous anastomoses passed this region. Remnants of the vestibular membrane were irregularly folded at the site of attachment. There were no large influences on the organ of Corti. However in the area of the lesion the stereocilia in the second row of outer hair cells appeared comparatively distorted or absent.

**Electrophysiology** The  $1 \mu\text{V}$  isopotential contour for this animal showed a loss of sensitivity in the mid frequencies between 500 and 2000 Hz (Fig 5). A maximum loss of 35 dB occurred at 1000 Hz. The input-output function at 4000 and 10000 Hz appeared to be within the normal (Figs 6 and 7). There was a significant reduction in the input-output function at 2000 Hz (Fig 5).

#### Animal No 4 (338)

**Surgery** A small fistula was made in the scala tympani. Moderate leakage of perilymph was noted. Two collecting venules were divided and there was approximately 10 seconds of bleeding.

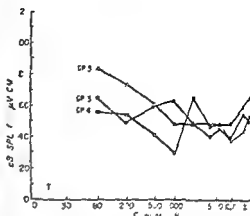


Fig 12 Animals 3 4 5 (344 338 323) Change in  $1 \mu\text{V}$  sensitivity contour following lesion in the scala tympani. The shaded area shows 2 SD about mean curves for a control group of animals

**Morphology** The membranous external wall was detached from the bone at the lesion and bulged somewhat medially. The lesion in the basal part of the scala tympani was completely healed by bone. On the inside of the external wall there was an accumulation of blood at the site of lesion and two or three large collecting venules and corresponding finer branches were uninjected. The vascular pattern of the scala vestibuli and media was in all respects normal.

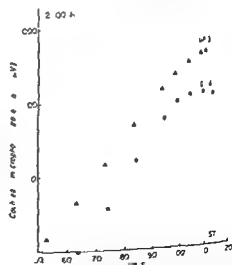


Fig 13 Animals 3 4 5 (344 338 323) Input-output function of the cochlear microphonics at 2000 Hz lesions made in scala tympani. The shaded area represents 2 SD about the mean value for a control group of animals

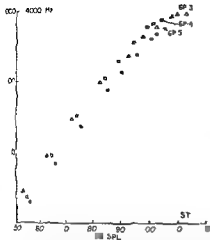


Fig. 14 Animals 3 4 5 (344 338 323) Input-output function of the cochlear microphonic at 4000 Hz for lesion made in scala tympani. The shaded area represents SD about the mean value for a control group of animals.

was the pattern in the scala tympani except the site of the lesion.

**Electrophysiology.** The 1  $\mu$ V isopotential behaviour was normal for both low and high frequencies (Fig. 12). The only frequency in which a significant difference occurred was at 2000 Hz. The loss at 2000 Hz was 22 dB. The input-output function at 2000 Hz (Fig. 13) showed a markedly reduced dynamic range. The input-output functions at 4000 Hz (Fig. 14) showed a slight change

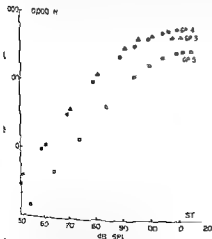


Fig. 15 Animals 3 4 5 (344 338 323) Input-output function of the cochlear microphonic at 10000 Hz for lesion made in scala tympani. The shaded area represents SD about the mean value for a control group of animals.



Fig. 16 Animal 5 (323) External wall basal turn apico-basal section. The lesion (framed area) affects a large collecting venule in the basal part of the scala tympani (ST) (see also Fig. 18). Almost all collecting venules (CVL) are well injected corresponding to the lesion. Only two show deficient contrast injection (see Fig. 17).

in the slope of the function but the maximum amplitude remained within that seen in the control animals. The input-output function for 10000 Hz was within normal limits (Fig. 15).

#### Animal No 5 (323)

**Surgery.** An elongated fistula was made basally in the scala tympani. Moderate leakage of perilymph was noted. Three collecting venules were divided mechanically. There was approximately 20 seconds of bleeding into the scala.

**Morphology.** The lesion appeared to be completely healed by bone. The lesion affected one of the largest branches of the vein of the round window (Fig. 16). There was very little influence on the collecting venules in this region. Some collecting venules appeared very clearly closed down at the Y formed junction of two branches and the empty continuing channel was easily observed (Fig. 17). The large collecting

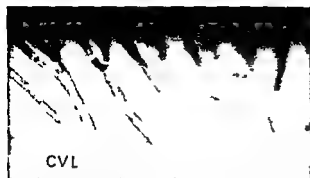


Fig 17 Animal 5 (323) Scala tympani basal turn apico-basal section. Corresponding to the lesion most of the collecting venules (CVL) are well contrast injected. Only two collecting venules apically to the right of the lesion show uninjected common parts where two branches unite.

venule in the middle of the lesion had a fairly uneven wall and large diameter (Fig 18). Single, uninjected radiating arterioles were also noted in the scala vestibuli. In the scala media, there appeared to be no influence on the vessels. The stria vascularis was fairly well injected and so was the vessel of the spiral prominence. There were no gross changes in sensory or neural structures.

**Electrophysiology.** The isopotential curve (Fig 12) indicated a decrease in sensitivity for frequencies below 1 500 Hz with the maximum loss of sensitivity occurring at 1 000 Hz. The magnitude of this loss was approximately 16 dB. The higher frequencies likewise showed decreases in the  $1 \mu V$  sensitivity; however, the



Fig 18 Animal 5 (323) Scala tympani basal turn apico-basal section. The lesion affects one large collecting venule. The vessel lumen is intact but lacks the normal smooth appearance.

order of magnitude is less than that in the lower frequencies. Input/output function at 4 000 and 10 000 Hz (Figs 14 and 15) showed a slight decrease in the dynamic range as compared to the control series of animals.

#### Animal No 6 (322)

**Surgery.** A wide exposure was made of the basal turn of the cochlea. A fistula was made extending from the basal part of the spiral segment almost to the level of the scala vestibuli. A microelectrode was introduced into the stria vascularis and 20  $\mu A$  were passed through the electrode for 3 seconds. This electrolytic lesion was subsequently repeated immediately adjacent to the site of the first lesion.

**Morphology.** At the site of the lesion, there was a heavy bony overgrowth to the bulla wall. This overgrowth originated in the middle of the stria vascularis and appeared to include the second and third turns. A small hole remained in the external wall at the lesion. An obvious gap in the vascular pattern of the external wall was noted with a corresponding segmental degeneration of the nervous structures in the spiral lamina (Fig 19). There was a pigment deficiency and reduced staining



Fig 19 Animal 6 (322) External wall, apico-basal section. The electrolytic lesion affected the scala vestibuli (SV) and scala tympani (ST) to a limited extent and the scala media (SM) to a great extent. Vessels are well injected on both sides of the lesion where there is a segmental narrowing gap in the vasculature.

the external wall in this region due to atrophy and thinning out of the tissues. The peripheral marginal vessels in the spiral lamina are less contrast injected at the level of the lesion than elsewhere. Sensory and supporting structures of the organ of Corti were completely degenerated in the area of the lesion. In this region, the basilar membrane was covered with squamous epithelium tissue. Likewise, a few nerve fibers were seen to extend in an aberrant course across the basilar membrane. Phagocytic cells were also evident (Fig. 20). Degeneration of afferent myelinated nerve fiber in the modiolus is evident (Fig. 21).

**Electrophysiology** The 1  $\mu$ V isopotential curve for this animal showed a significant increase in sensitivity of the CM as a function of frequency (Fig. 22). The greatest loss occurred at 1 000 Hz. The magnitude of loss at this frequency was approximately 100 dB. The average loss as a function of frequency was 50 dB.

Due to the magnitude of the loss, it was not possible to obtain input-output functions for this animal. (The sound pressure level required exceeded capabilities of the transducer.)

## DISCUSSION

Similarly to the previous investigation of acute animals, the current investigation presents



Fig. 20 Animal 6 (322) Spiral lamina basal turn transverse section. The organ of Corti adjacent to the lesion is absent. A few nerve fibers seem to extend in an aberrant course over this region. Phagocytes are seen here. Focusing at a lower level squamous epithelial "scar" tissue is seen covering the basilar membrane.



Fig. 21 Animal 6 (322) Spiral lamina basal turn transverse section. Adjacent to the lesion there is a segmental degeneration of sensorineural and supporting structures. There is a complete degeneration of the organ of Corti and myelinated afferent fibers in the modiolus. The vessel injection is slightly impaired centrally and much impaired peripherally in this segment.

observations on only a few animals. Reservations concerning conclusions based upon such a small sample must be raised. Some findings were similar and many were different from the previous examination.

It was possible to make both large and small mechanical lesions in the external wall of the guinea pig cochlea without necessarily inducing labyrinthitis (one diffuse, one circumscribed in the thirteen animals). High power magnification of the operating microscope allowed easy visualization of the pigmentation of the stria vascularis and the borders between the three

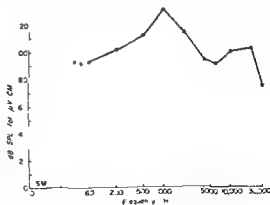


Fig. 22 Animal 6 (322) Change in the 1  $\mu$ V sensitivity contour following electrolytic lesioning of the scala media. The shaded area shows 2 SD about the mean for a control group of animals.

scalae of the external wall during surgery. With the mechanical lesions, there was often some leakage of perilymph and short term hemorrhage, which under visual observation always subsided within a minute or two. In the only case of the present material where electrical current was used for the lesion, the tissue seemed to be coagulated and there was no visible bleeding. This lesion had a considerably more pronounced effect. All mechanical lesions were healed by bone. In the case of the electrolytic lesion, there was a remaining hole in the cochlea which was sealed only by fibrous tissue. With either of these approaches, anatomically discrete lesions may be made in the cochlea.

In most animals the bony structures in the bulla wall and the cochlear wall showed a pronounced tendency to heal. Often the bony healing was abnormally thickened with bone protruding from the cochlea and also in many cases bony bridges formed between the bulla and the cochlear wall. In all cases except one, the intracochlear structures of the external wall had assumed approximately their normal position.

Findings of this study indicate that restricted mechanical damage to the cochlea results in restricted histopathology and a restricted effect on cochlear function. These results in the chronic preparation (6 weeks survival post-surgery) corroborate previously reported anatomical and electrophysiological findings on the short-term effects of such lesions (Hallén et al., 1974) and long term anatomical findings (Axelsson & Hallén, 1973).

The histological finding of contrast present in the cochlear vessels indicates the presence of a patent vascular lumen. It must be emphasized that the finding of contrast does not necessarily indicate a normal circulation in vivo since the contrast injection constitutes an artificial means of demonstrating the vessels. Our experience from both injected and uninjected vessels, however, has convinced us that there is a relationship between the outcome of the contrast injection and the *in vivo* condition of the vasculature. The effects on the vasculature were almost invariably limited to the external wall. The

findings in the present investigation by comparison to short term observations in the acute preparation, indicate that some vascular healing process follows these lesions. In the short-term experiments, scala vestibuli lesions always caused a gap in the vasculature, both centrally and peripherally to the lesion. In the present investigation, the three animals with scala vestibuli lesions showed only restricted influence on the vasculature at the site of the lesion and its immediate vicinity. In one of these animals, there was an additional thinning of a narrow segment of the spiral ligament with some influence on the vessels in the stria vascularis. In the other two, both the basal part of the scala vestibuli and the scala media demonstrated an approximately normal appearing vasculature.

The only scala media lesion of the present investigation was the electrolytically induced lesion. This injury was considerably larger both initially and in its end result. The effect of this lesion on cochlear vasculature was similar to the large mechanical lesions presented previously (Axelsson & Hallén, 1973).

The two scala tympani lesions had smaller effects on the vasculature and were similar to the effects observed in the acute preparation. There was no influence on the vasculature in the scala vestibuli or scala media. It thus appears that large lesions do not show secondary complete vascular healing, but that they show a segmental limitation with the effect on the vasculature limited to a narrow gap (Axelsson & Hallén, 1973). With small lesions, as in the present investigation, and a fairly short survival time, the vascular repair process seems considerably more pronounced and the lesions are not only limited in segmental space but also limited to the immediate surroundings of the lesion. These findings indicate a surprising healing tendency in the sensory organ.

These small lesions had less histopathological effect on the sensorineuroepithelium and supporting structure of the organ of Corti than expected. It was only in the case of the electrolytic lesion that clear degeneration occurred.

ed in this case, in addition to the effect on structures of the organ of Corti, the extent of myelinated fiber degeneration was quite dramatic. In all animals receiving the mechanical lesions alone, the only indication of some effect on organ of Corti structures was occasional distortion or absence of stereocilia in outer cells.

On the basis of the electrophysiological observation, five points may be discussed.

(1) There appeared no consistent relationship between individual lesions primarily restricted to one scala and subsequent electrophysiological effects. For example, similar lesions restricted to the scala vestibuli resulted in different degrees of electrophysiological depression. In general, no exact correlation was observed between the form and extent of the electrophysiological depression and specific features of the form and extent of histopathological changes. These observations hold for both animals examined acutely, immediately after surgery (Hallen et al., 1974) and chronically, following a 6-week survival time. This lack of relationship cannot simply be attributed to some immediate generalized disruption of cochlear function. Our inability to determine the exact relationship between anatomical and electrophysiological changes induced by these lesions may, however, simply be due to the small sample size examined to date.<sup>1</sup>

It is clear that on a gross level, a relationship exists between organ of Corti destruction and depression of the cochlear response. The most dramatic change in this study was when a microelectrode was introduced into the scala media and an electrolytic lesion created. A dramatic decrease was seen in the  $1 \mu\text{V}$  isopotential contour. This occurred for all frequencies. Also, morphological findings were

Reservations regarding the sensitivity of the measures used in these studies were discussed in the previous paper (Hallen et al., 1974). In particular, as suggested by others (Bohne et al., 1973; Bredberg, 1968) we feel low power evaluation of organ of Corti structures permitted by light and phase contrast microscopy is insufficient sensitivity to reflect the functional state of these structures except in the case of marked destruction.

severe within the area adjacent to the lesion where a complete elimination of the organ of Corti and the development of epithelial scar tissue along the basilar membrane was observed. It is difficult to compare this scala media lesion with the mechanical lesions placed in the other scalae in this group of animals. However, the observation of a greater effect with the scala media lesion than observed with lesions of the other scalae, is consistent with our findings on the short term effects of such surgeries. It is also consistent with the findings of Tasaki et al. (1952). The only condition under which they noted changes in the electrophysiology of the cochlea was when lesions were made in the scala media. We suggest that this may be due to the fact that lesions in the scala media have a direct effect on the longitudinal distribution of the CM in the cochlear partition. Certainly in regards to other investigations on the generation of the CM (Honrubia & Ward, 1968), such an explanation is reasonable.

(2) In assessing cochlear function, it appears that the input-output function of the CM may be more sensitive to cochlear changes than an examination of the  $1 \mu\text{V}$  isopotential contour. This was a consistent observation in animals examined in this study. These observations agree with previous findings of the effects of experimental manipulation on cochlear activity (McPherson & Miller, 1972, 1974).

(3) By comparison to observations on the immediate effects of mechanical lesions to the lateral wall of the cochlea (Hallen et al., 1974), the somewhat longer term effects examined in this investigation appear somewhat more restricted. Again, limited sample size employed in these two studies places some reservations on such comparisons.

(4) One of the most important observations in this study is the fact that healing occurred after discrete lesioning of the cochlear wall, resulting in relatively small changes in the electrophysiological response of the cochlea. This is particularly encouraging in that the changes seen electrophysiologically in this study were less severe than those seen where the short-

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term electrophysiological effects of such lesions were examined. Encouraging also is the continued observation of the restricted nature of anatomical and electrophysiological effects of such restricted lesions.

(5) Only small electrophysiological changes occurred in association with those surgeries in which slight perilymphatic leakage and/or bleeding was encountered. However, moderate or excessive bleeding and leakage was associated with a clear and, in many cases, severe depression of the cochlear potentials. Apparently, perilymphatic leakage at the time of surgery, in which the only cochlear damage was a small mechanical lesion of the lateral wall, may serve the surgeon as a cue for both the immediate and more long term surgical influence on cochlear function.

It should be clear that we place many reservations upon the observations made in this study, principally because of a small sample size of subjects examined with this approach to date. Additionally, one must be concerned about the sensitivity of such measures as round window recordings of electrical activity of the cochlea and light microscopy for the examination of histopathological changes. However, we do suggest that the combined electrophysiological and histopathological approach taken in this study is a fruitful one for studies of this nature. We feel that at this time it is important to determine the effect such inner ear surgery has on the status of the cochlea. Such observations are significant in light of the increasing interest by otolaryngologists on the possibility of performing vascular and electrode implantation surgeries on the cochlea of man.

## ZUSAMMENFASSUNG

Die chronisch morphologischen und elektrophysiologischen Einwirkungen von kleinen Schäden bei sechs Meerschweinchen werden mitgeteilt. Die Schäden wurden an der äusseren Wand in einer der drei Scala von der basalen Schneckenwindung gemacht. Sechs Wochen später wurde das runde Fenster mit Hilfe eines postaurikulären Zuganges exponiert und eine Platinum-Iridium-Ball-Elektrode wurde am Fenster angelegt. Messungen der CM wurden mit 10 Frequenzen vorgenommen. Nach den elektrophysiologischen Messungen wurden die Tiere

mit Berlinerblau perfusiert und die Temporalnerven wurden ausgenommen, fixiert und gefärbt, et al., und für Mikroskopie präpariert. Im allgemeinen waren die elektrophysiologischen Antworten von der Cochlea nach den mechanischen Schäden niedriger. Morphologisch wurden nur wenige und immer segmental begrenzte Einwirkungen an den Gefässen in der Gegend des Schadens beobachtet. Das Cortische Organ war histopathologisch sehr wenig beeinflusst.

Die Befunde dieser Untersuchung deuten darauf, dass begrenzte mechanischen Schäden an der Cochlea auch begrenzte histopathologische Effekte und eine begrenzte Einwirkung auf die Cochleafunktion ergaben. Keine regelmässige Verhältnisse zwischen mechanischen Schäden und der zu einer Scala begrenzten und die darauf folgenden elektrophysiologischen Effekte wurden beobachtet. Dagegen scheint es als ob ein Zusammenhang zwischen Destruktion vom Cortischen Organ und Depression der cochleären Antworten auf elektrolytische Schäden in der Scala media existiert.

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# SYNAPTIC VESICLES IN THE COCHLEA

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(Received February 15, 1974)

**Abstract** The cochlea is innervated by afferent, efferent and sympathetic neurons. The afferent synapses and the efferent and sympathetic nerve terminals contain transmitter substances within their synaptic vesicles. These transmitters are likely to be chemically different in each of the three cochlear nerve populations. The character of intra-cochlear synaptic vesicles was investigated in order to throw some light on transmitter identity. Their staining properties were examined using different fixatives and after pretreatment with false transmitter substances and a monoamine depleting drug. The efferent and sympathetic cochlear terminals were identified and differentiated by surgical ablation of either the efferent or sympathetic bundle or by cervical sympathectomy. The nature of these terminals could also be ascertained in separated animals but only after pretreatment with false transmitters or after special fixation techniques. In this way a catecholamine appeared to be the transmitter of the sympathetic nerve-terminals. The staining properties of cochlear afferent synaptic vesicles and synaptic terminals made it unlikely that a catecholamine was the transmitter. Vesicles in efferent terminals reacted similarly to vesicles in cholinergic neurons.

(1961) and Richardson (1962, 1966). Small vesicles with a diameter of about 500 Å and large vesicles around 1 000 Å in diameter can occur together.

Electron microscopic evidence now exists that catecholamines are contained in dense-cored vesicles (Richardson, 1962, 1966). This has been confirmed by Hökfelt (1968) using an *in vitro* technique involving incubation in monoamine-containing solutions and fixation with potassium permanganate. There is good evidence that both large and small dense core vesicles in adrenergic axons contain a monoamine or its precursor (Klein & Turesson-Klein, 1971). Administration of false transmitters and the action of certain drugs has verified this conclusion. Cholinergic neurons also contain large dense cored vesicles but they are not affected by the above-mentioned substances and are not considered to be involved in the monoamine metabolism. It is thought that these vesicles as well as so called coated vesicles and large clear vesicles, which also exist in most nerve endings, are involved in the process of recovery of membrane and related material (Akert & Sandri, 1970; Heuser & Miledi, 1971).

The complicated innervation pattern in the cochlea and organ of Corti has been extensively studied. From the electron microscopic work of Engström (1958) and Engström & Wersäll (1953, 1958) two types of nerve ending, the vesiculated and the less vesiculated were identified. Vesiculated nerve endings were assumed to be efferent in nature (Engström, 1958). The path-

The existence of vesicles in the presynaptic part of neurons was first discovered by De Robertis & Bennett (1954) and Palade & Palay (1954). Since then several types of vesicle have been described. Most nerve endings contain small clear vesicles clustered in the vicinity of the presynaptic membrane. They are found in both cholinergic neurons and in neurons containing other transmitters, for example biogenic amines and amino acids.

A special type of synaptic vesicle containing a dense core was discovered by Hager & Tafuri (1959), De Robertis & Pellegrino De Iraldi

The investigation has been supported by grants from the Medical Research Council, no. 04x 2461, and the Karolinska Institute.

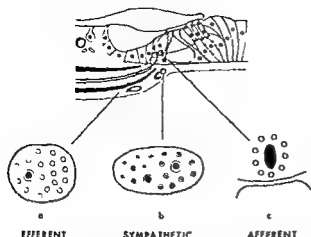


Fig. 1 Schematic drawing illustrating typical location for synaptic vesicles of different types (a) efferent terminals of the olivo-cochlear bundle (b) sympathetic nerve endings (c) the afferent synapse between inner hair cells and sensory nerve fibre

ways of the efferent innervation were originally described by Rasmussen (1946, 1960) in cat and has also been identified in the rabbit (Borg, 1973). Further investigations on the efferent innervation in the cochlea have been performed by Kimura & Wersäll (1962), Smith & Rasmussen (1963), Spoendlin & Gacek (1963) and Iurato et al. (1971).

The cochlea also receives a sympathetic innervation, which can be demonstrated with the Falck-Hillarp (Falck et al., 1962) fluorescence technique for catecholamines (Terayama et al., 1965, 1966; Vinnikov et al., 1966; Spoendlin & Lichtensteiger, 1966, 1967) and by electron microscopy (Densert, 1974). As far as the afferent nerve fibres are concerned the hair cells are the presynaptic elements—they contain synaptic vesicles at their base and are likely to transmit chemically.

The aim of the present study was to investigate synaptic vesicle populations in the organ of Corti by using surgical elimination and different fixation methods in order to find distinguishing features which can facilitate the identification of different synapses and terminals, and perhaps throw some light on the question of transmitter identities.

Particular attention was paid to efferent

and sympathetic, adrenergic nerve endings in the region of the osseous spiral lamina and the afferent synapse at the base on hair cells.

## MATERIAL AND METHODS

Fourteen white rabbits weighing from 1.2 to 3.0 kg were included in the present investigation. The following procedures were used.

### A. Surgical Procedures

#### 1. Sectioning of the olivo-cochlear bundles

In 3 animals, under Nembutal® anaesthesia, the skull was opened in the occipital region. The cerebellum was elevated slightly. Using a skin knife, at an angle of 45° to the fourth ventricle a 2–3 mm deep incision was made through the restiform body, immediately behind the dorsal cochlear nucleus. Both the crossed and uncrossed olivo-cochlear fibres, as well as the reticocochlear fibres, were thereby interrupted as they passed along the intracerebral part of the vestibular nerve. Bilateral lesions were produced. Five to 9 days after sectioning the olivo-cochlear bundle, the animals were decapitated and the brain stem was removed and fixed in 10% formalin. After embedding in paraffin, the specimens were sectioned and stained according to Klüver & Barrera. The cochleas were fixed in osmium tetroxide as described below (B1) or by potassium permanganate (B2).

#### 2. Sympathectomy

In 2 animals unilateral extirpation of the superior cervical ganglion was performed under Nembutal® anaesthesia 1 and 2 weeks before sacrifice. In 2 other animals, where the olivo-cochlear bundle was sectioned, unilateral cervical sympathectomy was also performed. 5 OH DA as described under C1, was administered to each animal in each group before decapitation.

### B. Fixatives Used

1. 1% osmium tetroxide in veronal acetate buffer (Rhodin, 1954), post mortem or *in vivo* perfusion *in vivo*.



2 A low power view of a section through the brain showing bilateral surgical lesions through the olivohypocretal bundle. Stained according to Kluver and Barrera.

3% potassium permanganate in 0.1 M sodium phosphate buffer at pH 7.0 for 45 minutes (Luft, 1956, Richardson, 1966, Hokin, 1968).

3% glutaraldehyde (0.133 M sodium phosphate buffer, pH 7.3) followed by 1% osmium tetroxide.

### C Pretreatment with Drugs

The false transmitter 5-OH DA (3,4,5-trihydroxyphenylethylamine hydrochloride) was administered intravenously or by local perfusion as described under B2. 5-OH DA was administered i.v.  $4 \times 20$  mg/kg over a period of 4 hours (Tranzer & Thoenen, 1967). For local perfusion the tympanic bulla was opened and a small hole was made to the scala tympani with the aid of a diamond drill. The stapes was removed and the cochlea was perfused via the drilled hole for 5 minutes with a Ringer solution containing 0.1 mg/ml 5-OH DA.

2 6-OH DA (2,4,5-trihydroxyphenethylamine hydrochloride) was administered i.v. or via local perfusion before fixation in osmium tetroxide according to B1. 6-OH DA 50 mg/kg was administered i.v.  $\frac{1}{2}$  to 3 hours before sacrificing the animals. Local perfusion with 6-OH DA was performed in the same way as for 5-OH DA, but to the solution was added 0.2 mg/ml ascorbic acid.

3 Reserpine (10 mg/kg) was administered 24 hours before sacrifice. Fixation was performed in potassium permanganate or glutaraldehyde and osmium tetroxide according to B2 and B3 respectively.

Fixed specimens were dehydrated in ethanol and embedded in Epon (Luft, 1961). Ultrathin sections were cut with a diamond knife on an LKB ultratome. The sections were stained in uranyl acetate (Reynolds, 1963) and lead citrate (Watson, 1958) except for specimens fixed in potassium permanganate which were only briefly stained in lead citrate. The sections were examined in a Siemens Elmiskop I electron microscope.

## RESULTS

### *Surgical Procedures*

When the ipsilateral superior cervical ganglion was removed all adrenergic nerve terminals and fibres in the cochlea degenerated. One week after the sympathectomy no adrenergic structures were identified around the spiral vessel of the tympanic lip and in the habenula region, where they were normally abundant in untreated animals (Fig. 1). In other parts of the cochlea there were no signs of nerve fibre degeneration.

When the olivo cochlear bundles were sectioned (Fig. 2) all vesiculated nerve endings of efferent nature under the inner and outer hair cells, like nerve fibres in the inner spiral bundle and tunnel spiral bundle (Figs 3, 5) degenerated. About 2 weeks later it was not possible to identify any efferent axons in the cochlea (Figs 4, 6).

In two experiments unilateral sympathectomy was performed in addition to bilateral total olivo-cochlear ablation. The cochlea on the sympathectomized side did not contain any vesiculated endings, whereas the remaining vesiculated nerve endings in the cochlea on the other side were of typical adrenergic appearance and location.

### *Vesicle Appearance in Different Fixatives*

#### *Osmium tetroxide*

Fixation in 1% osmium tetroxide gave good preservation of ultrastructural details in the cochlea. Efferent and adrenergic nerve terminals were filled with synaptic vesicles having similar appearance (Figs 7, 8). Thus, it was not possible to differentiate between adrenergic and efferent terminals according to the number or size of synaptic vesicles or by other ultrastructural features. The afferent synapse in hair cells also contained this type of vesicle (Fig. 15). Most vesicles had a diameter of about 300–400 Å, although some were as large as 1 000 Å in diameter. All synaptic vesicles were spherical. As a rule, small vesicles appeared devoid of electron dense material, while large vesicles in both efferent and adrenergic terminals contained a rather dark, electron dense core. The

synaptic bar at the afferent synapse was also electron dense. A few adrenergic synaptic vesicles might contain a small dense core. These cored vesicles were more apparent when specimens were fixed by *in vivo* perfusion of fixative. There was no apparent difference in properties of efferent terminals when fixation was performed either *in vivo* or minutes after death.

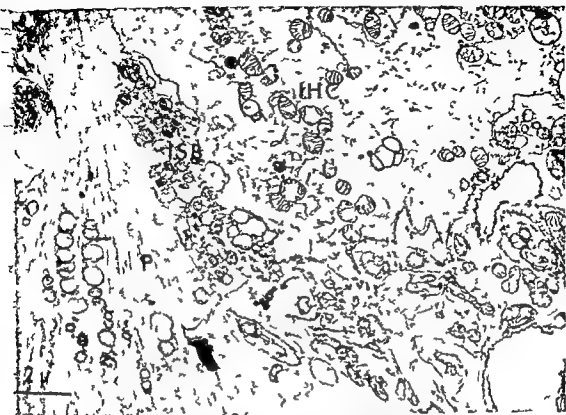
#### *Glutaraldehyde*

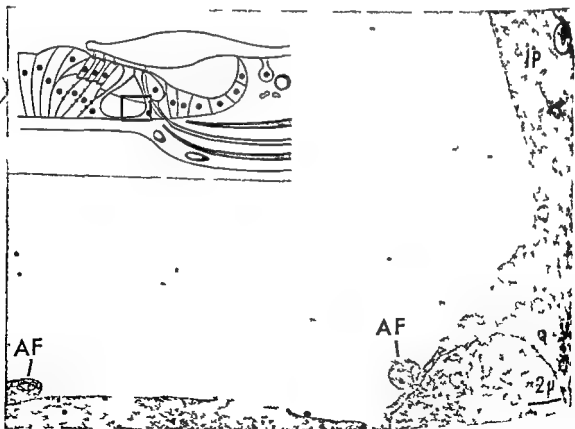
When the specimens were fixed in glutaraldehyde and postfixed in osmium tetroxide, the synaptic vesicles in efferent endings and cells appeared empty and were devoid of electron dense material (Fig. 9). A few monoamine storage vesicles contained a dense core (Fig. 10). Large synaptic vesicles contained a distinct dense core, both in efferent and adrenergic terminals. The density of the synaptic bar in hair cells was similar to these dense cores. The electron dense material almost filled the vesicles, leaving only a narrow gap between the dense core and vesicular membrane. Large dense-cored vesicles were abundant in both inner spiral bundle below inner hair cells and the tunnel spiral bundle.

#### *Potassium permanganate*

This is a strong fixative and the preservation of ultrastructural detail was inferior to that obtained with osmium tetroxide. However, synaptic vesicles and terminals were fairly well preserved. In efferent endings small vesicles appeared empty and the large ones contained an electron distributed weakly staining material (Fig. 11). Adrenergic terminals in rather large number

Fig. 3 Electron micrograph from a normal animal (10000 $\times$ ) showing the afferent synapse in the inner pillar cell (TSB). The synaptic bar is electron dense and the synaptic vesicles are electron clear. The spiral bundle is indicated by an arrow. The inner pillar cell is indicated by a star.





les contained a small but distinct dark core. 12) These dense cores were most evident in adrenergic vesicles. Small vesicles in efferent nerve terminals were devoid of an electron dense core. Similarly, afferent synaptic vesicles did not contain a dense core and the synaptic bar was barely visible.

### Pretreatment with Drugs

#### *Droxydopamine*

When the animals were pretreated with 5-OH-DA, accumulation of the substance was seen in adrenergic synaptic vesicles. After fixation in potassium permanganate a distinct dense core appeared in all monoamine-storing vesicles. 13) Efferent synaptic vesicles, on the other hand, did not change after pretreatment and no change in electron opacity was observed. Vesicles at the afferent synapse did not contain a dense core, nor did the synaptic body stain intensely (Fig. 14). The appearance of the afferent synapse in an untreated animal is shown in Fig. 15.

After fixation in glutaraldehyde and osmium tetroxide adrenergic synaptic vesicles contained an electron dense substance. This black substance appeared in both small and large vesicles. Large synaptic vesicles in efferent terminals contained a distinct core but its electron density was not increased by 5-OH-DA pretreatment.

#### *Droxydopamine*

When 6-OH-DA was administered i.v. it accumulated in adrenergic synaptic vesicles. Within a few minutes most vesicles exhibited a dense core. After fixation in osmium tetroxide. Administration of 6-OH-DA, however, was followed by

a degeneration and the number of dense cored vesicles rapidly diminished with time. The accumulation of 6-OH-DA in adrenergic vesicles was even more evident when the cochlea was perfused locally with a 6-OH-DA-containing solution (Fig. 11). In efferent nerve terminals there was no visible accumulation of 6-OH-DA, nor were hair cells affected.

#### *Reserpine*

When animals were pretreated with reserpine the dark core of adrenergic vesicles did not appear after fixation in potassium permanganate (Fig. 17) and it was no longer possible to distinguish vesicles in adrenergic terminals from those in efferent endings or hair cells. No change was seen in the appearance of the synaptic bar in inner hair cells.

## DISCUSSION

Transmitter substances and their identification by electron microscopy has attracted much interest since the discovery of storage vesicles in the presynaptic part of neurons (De Robertis & Bennett, 1954; Palade & Palay, 1954). The theory that acetylcholine is stored in vesicles was put forward by Del Castillo & Katz (1956). von Euler & Hillarp (1956) proposed a similar hypothesis concerning the storage of noradrenalin. Considerable effort has been made to identify transmitter substances with electron microscopy. Such identification has been possible only for biogenic amines.

The ability to demonstrate monoamines at an ultrastructural level depends primarily on the fixation technique. Osmium tetroxide was first used to demonstrate amine storage sites in peripheral neurons (Grillo & Palay, 1962; Richardson, 1962). Potassium permanganate, which was introduced as a fixative by Luft (1956), has turned out to be specific in demonstrating biogenic amines (Richardson, 1966). The reliability of this technique was further improved by Hökfelt (1968). The reaction between the fixative and the amine complex results in a precipitate, which

5 Electron micrograph from a normal animal, showing efferent nerve fibres travelling in the tunnel of the olivocochlear bundle (TSB) and as tunnel crossing fibres (CF) in efferent fibres (AF) cross the tunnel at a lower level. IP, inner pillar cell.  $\times 8400$ .

6 After transectioning the olivocochlear bundle, efferent fibres disappear, whereas afferent ones remain.  $\times 6200$ .





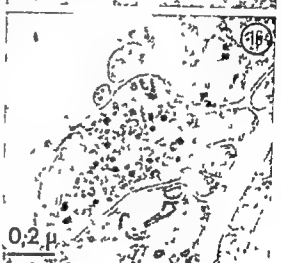
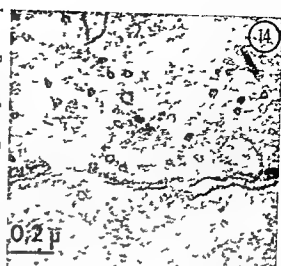
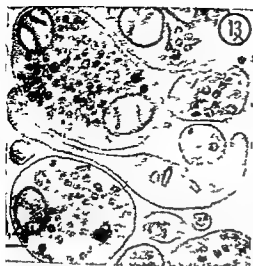


Fig. 13 Pretreatment with 5 hydroxydopamine and fixation in potassium permanganate enhances the density of the core in adrenergic synaptic vesicles  $\times 43\,500$

Fig. 14 The synaptic vesicles in the afferent synapse fail to show a dense core in spite of pretreatment with hydroxydopamine and fixation in potassium permanganate. The synaptic body is weakly stained  $\times 53\,300$

Fig. 15 The afferent synapse in a non treated animal is shown after fixation with osmium tetroxide  $\times 51\,300$

Fig. 16 Adrenergic nerve endings in a cochlea perfused with a 6-OH DA solution. Vesicles contain a black substance and degeneration has started. Fixation in osmium tetroxide  $\times 59\,000$

Fig. 7 Efferent terminals fixed with osmium tetroxide. Small vesicles and a few large dense-cored vesicles  $\times 25\,700$

Fig. 8 Adrenergic terminals fixed with osmium tetroxide. Vesicles similar to efferent ones  $\times 27\,700$

Fig. 9 Fixation in glutaraldehyde followed by osmium tetroxide. Efferent terminals contain both small clear and large dense-cored vesicles  $\times 31\,300$

Fig. 10 Fixation in glutaraldehyde followed by osmium tetroxide. Adrenergic terminals have a few small granulated vesicles. The large vesicles contain a dense core  $\times 37\,100$

Fig. 11 Potassium permanganate fixation does not alter the staining properties of efferent synaptic vesicles  $\times 41\,100$

Fig. 12 Potassium permanganate stains synaptic vesicles in adrenergic terminals in a characteristic manner. Small as well as large vesicles have a dense black core  $\times 37\,300$

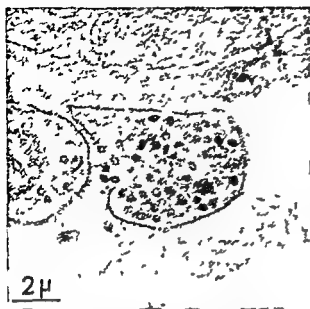


Fig 17 Pretreatment with reserpine depletes catecholamine stores in adrenergic synaptic vesicles and they no longer exhibit a dense core  $\times 54\,000$

is visible in the electron microscope as a dark, electron dense core in the amine storage vesicle. The use of 'false' transmitter substances such as  $\alpha$  methylnoradrenalin (Bondareff, 1965, Hökfelt, 1968) and 5 OH DA (Tranzer & Thoenen, 1967) made it possible to increase the amine level in amine storage vesicles and make the demonstration of adrenergic neurons more reliable. It has been established that small dense-cored vesicles in adrenergic neurons contain a monoamine and that the large dense cored vesicles take part in monoamine metabolism. The existence of large dense cored vesicles in cholinergic neurons has been interpreted in different ways. However, there is no evidence that monoamines are present in cholinergic neurons.

In the present study the location of efferent and adrenergic nerve endings was established by surgical ablation of the olivo-cochlear bundles and by cervical sympathectomy. The identification of the afferent synapse posed no problem.

In the ultrastructural study of the inner ear, fixation with osmium tetroxide or glutaraldehyde followed by fixation in osmium tetroxide are the most commonly used techniques. These methods

give good ultrastructural preservation but efferent and adrenergic nerve terminals fixed with these agents, have almost the same appearance (Ross, 1973). After fixation in glutaraldehyde and/or osmium tetroxide a few small adrenergic vesicles contain a black core but this is not sufficient to ascertain the identity of the terminals. When the specimens were fixed in potassium permanganate most amine storage vesicles appeared granulated, and their appearance was enhanced after 5 OH DA pretreatment. The existence of a monoamine in these granulated vesicles was further proved by their disappearance after pretreatment with reserpine.

Synaptic vesicles in efferent nerve endings in adrenergic nerve terminals and in afferent synapses in hair cells have been compared. The staining properties of efferent synaptic vesicles are as would be expected in cholinergic neurons, a fact which agrees with the suggestion that acetylcholine might be the transmitter in the olivo-cochlear neurons (Schuknecht et al 1969).

Nerve terminals in the habenula region and around the spiral vessel of the tympanic lip contain vesicles which have all the characteristics of catecholamine-containing neurons. The presence of fluorescence specific for catecholamines in this region supports this concept (Densez, 1974). Attempts have been made to identify transmitter substances in afferent synapses in hair cells. Three theories as to the nature of the afferent transmitter have been suggested: acetylcholine (Osborne & Thornhill 1972), glutamate (Steinbach & Bennett, 1971) and  $\gamma$  aminobutyric acid (Flock & Lam 1974).

Since afferent synaptic vesicles and bars stain weakly in potassium permanganate and are not influenced by 5 OH DA, 6 OH DA and reserpine it seems unlikely that a catecholamine is involved.

## ACKNOWLEDGEMENT

For generous supply of drugs (5 and 6-hydroxydopamine) we wish to thank Dr H. Corrod, Håssle, Sweden. The authors would also like to express their gratitude to Mrs. Marie Louise Spångberg and Mrs. Britta Flack for technical assistance and to Mr. Bengt Hedberg for excellent photographic work.

## ZUSAMMENFASSUNG

Die Cochlea wird von afferenten, efferenten und sympathischen Neuronen innerviert. Die afferenten Synapsen wie die efferenten und sympathischen Nervenendigungen haben Transmittersubstanzen in ihren synaptischen Vesikeln. Diese Transmitter sind wahrscheinlich chemisch verschiedene Substanzen in jeder der drei Nervenpopulationen. Die Beschaffenheit der intracochleären synaptischen Vesikel wurde in Hinblick auf die färbischen Eigenschaften unter Anwendung verschiedener Fixierungsmittel und nach Vorbehandlung mit diesen Transmittersubstanzen sowie einer Monoamin-oxidierenden Substanz untersucht. Die efferenten und sympathischen cochleären Nervenendigungen wurden durch chirurgische Abtrennung des efferenten olivocochleären Bündels oder durch cervicale Sympathektomie isoliert und differenziert. Die Natur dieser Nervenendigungen konnte auch an nicht operierten Tieren ermittelt werden, jedoch nur nach Vorbehandlung mit einem falschen Transmitter oder nach Verwendung von speziellen Fixierungstechniken. In solchen Fällen erschienen Catecholamine in sympathischen Nervenendigungen, waren aber in efferenten Nervenendigungen nicht zu sehen. Die färbischen Eigenschaften der cochleären efferenten synaptischen Vesikel und synaptischen Körper schieden die Annahme unwahrscheinlich, daß ein Catecholamin als Transmitter wirkte.

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## PINOCYTOSIS IN THE PILLAR CELLS OF THE ORGAN OF CORTI

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**Abstract** Electron micrographs are presented showing pinocytosis in the head plates of the pillar cells of the organ of Corti. The possibility of fluid transport between the intercellular spaces and the subtektorial space is inferred from these observations. The capabilities of the pillar cells for fluid transport were further examined through the use of the exogenous tracer, Thorotrast<sup>®</sup>, which was taken up in the tunnel-spaces until at least 40 min after reduction into perilymph. After 2 hrs Thorotrast was found in vesicles in all parts of the pillar cells and closely related to the external membrane. On the basis of a review of histochemical observations relating to the pillar cells together with morphological findings and results of tracer experiments, it appears that these cells are involved in active transport processes involving perilymph, endolymph, and the fluid of the subtektorial space. They help to maintain microhomeostasis in the cochlear fluid.

Recent studies have produced evidence that certain of the non sensory cells of the organ of Corti play important roles in the transport of ions and macromolecules (de Lorenzo et al, 1973, Lim, 1970b, Vosteen, 1970). During the course of a previous study involving close scrutiny of the reticular lamina in the guinea pig (Wright & Preston, 1973), we found tiny openings, frequently surrounded by dense clusters of microvilli, scattered on the head plates of the pillar cells. Further study of these structures through both scanning and transmission electron microscopy led to a series of exogenous tracer experiments designed to investigate the active

transport capabilities of the pillar cells. The results of this work, together with histochemical and ultrastructural data from other investigations, suggest that, in addition to their function as structural supports, the pillar cells are capable of transporting substances between fluid compartments in the cochlea by pinocytosis or cytotransport.

### METHODS

Our initial observations of openings in the pillar head plates were made in a series of 5 young adult guinea pigs (3 experimental and 2 control animals) used in a study of cochlear innervation (Wright & Preston, 1973). Cochleas from these animals were fixed immediately *post mortem* by the zinc iodide-osmium tetroxide (ZIO) method as described in the above publication. Subsequently, the same structures, identical in appearance, have been seen in normal guinea pig cochleas fixed in 6% phosphate-buffered glutaraldehyde, or 10% cacodylate-buffered acrolein, followed by post-fixation in 1% phosphate-buffered osmium tetroxide (OsO<sub>4</sub>). Both *in vivo* and rapid *post mortem* perfusion of the perilymphatic spaces was employed.

Freeze drying for scanning electron microscopy (SEM) was based on the technique of Bredberg et al (1970). CO<sub>2</sub>-critical point drying from amyl acetate-immersed, ethanol dehydrated tissue was also used as a check for freeze-drying artifacts. Ears from several mammalian species in addition to guinea pig were prepared

Support for this work was provided by NINDS, USPHS Intramural Fellowship NS 36,642, Research Grant NS 065, Program Project Grant NS 05785, and Training Grant NS 05679.

using similar techniques and surveyed for the presence of these structures in pillar cells

Colloidal thorin sol-dextrin (Thorotrast®) was introduced into the perilymphatic spaces by a method similar to that of von Ilberg (1968*a, b*). After surgical exposure of the cochlea both the round and oval windows were opened. The perilymph was then replaced by Thorotrast by perfusion through either the round or oval window.

Perilymphatic perfusion of Thorotrast was repeated several times during the course of each experiment. Large amounts of Thorotrast were presented to the perilymphatic scalae to facilitate interpretation of its distribution in scala media at the end of the experiment. Great care was exercised not to puncture the basilar membrane through the round window. To terminate the Thorotrast experiments we perfused the cochlea with 6% glutaraldehyde, decapitated the animal and immersed the temporal bones in the fixative for 24–48 hrs before  $\text{OsO}_4$  post-fixation, microdissection and Araldite-embedding according to conventional techniques.

## RESULTS

### *Electron-microscopic findings*

Initial observations with SEM disclosed small openings in the head plates of the pillar cells (Fig 1*a–e*). These structures vary greatly in size (0.5–3  $\mu\text{m}$  greater dimension) but are consistent in having smooth, raised borders, apparently composed of fusions of microvilli. Suggestions of internal structure, again interpreted as being microvilli, are frequently visible (Fig 1*a, f*). Raised areas, blister-like in appearance, can also be seen, some of these suggest underlying channels (Fig 1*a, b*). Other structures, with accompanying rings of fused microvilli, brought to mind openings either recently closed or soon to be opened (Fig 2). When we were able to look into the tunnel, after dissecting away the basilar membrane and mounting the specimen in inverted fashion, we again detected openings in the pillar cell surfaces (Fig 2).

Having convinced ourselves of the validity of these observations after using three different

primary fixatives and both critical freeze drying methods, we attempted to illustrate the structures using transmission microscopy (TEM). We have been so far in finding an actual opening, membrane-bound channel, lined with a pillar head plate is clearly shown.

Our current impression regarding distribution of the head plate openings in turns is that they probably occur uniformly from base to apex, although perhaps be more easily noticed in the third of the organ of Corti. The number in a given tissue sample varies widely from several per pillar cell to only a few in an entire cochlear turn. We have now structures in the chinchilla, guinea pig, tree shrew, and macaque (*Macaca*).

### *Experiments with Thorotrast*

The openings observed in pillar cells suggestive of pinocytotic activity, we undertook to learn more about the use of the pillar cells for fluid transport of an exogenous tracer, Thorotrast. A total of 7 experiments were done using this tracer. The first fixed 20 and 40 min and 2 hours after the administration of the tracer in a chinchilla, and 30 min, 2 hours (2 animals) and 4 hours in 4 guinea pigs.

In general the distribution of  $\text{ThO}_2$  particles observed with TEM in our experimental series is similar to that seen by von Ilberg (1968*a*) and by Duvall & Quick (1969). Large amounts of the tracer were visible, in all ears, in the stria of the basilar membrane, spiral ligament and spiral ligament and in vesicles in the

Fig 1 Scanning (*a–e*) and transmission (*f*) electron micrographs of the guinea pig organ of Corti showing structures in the tops of inner pillar cells thought to be openings. *a–e* 710 fixation, *f* 710 fixation, *g* 710 fixation, *h* 710 fixation, *i* 710 fixation, *j* 710 fixation. Bars indicate 1  $\mu\text{m}$ .





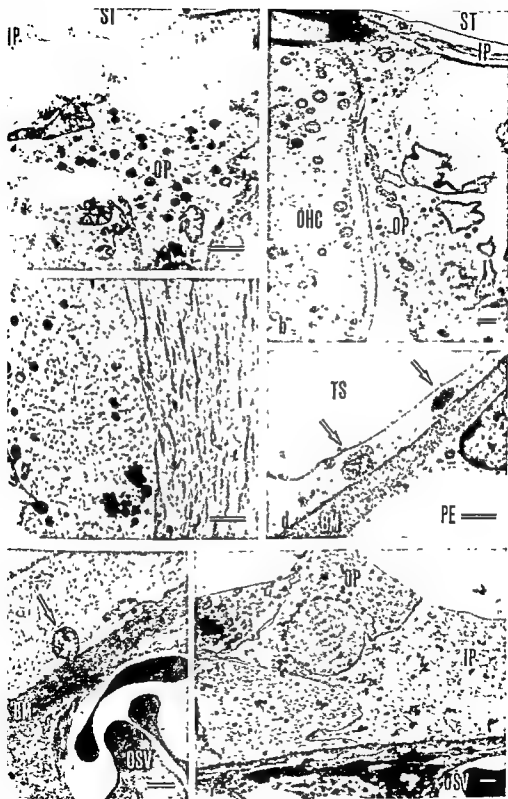


Fig 2 Scanning electron microscopic view of tunnel aspect of pillar heads showing openings (downward pointing arrows inset) in or between outer pillar cells. Upward pointing arrows indicate ring arrangements of

microvilli frequently seen in neighborhood of openings. Guinea pig third turn.  $\text{OsO}_4$  fixation. *IP* pillar cell. *OP* stalk of outer pillar cell.

Fig 3 Transmission electron micrographs of the organ of Corti apical turn from chinchillas showing the distribution of Thorotrast which had been injected into perilymph 2 hours (a-c) and 40 min (f) before fixation. Regions of pillar cells shown in each picture are described in the text. The arrows indicate large vesicles in the pillar feet containing high concentrations of tracer apparently

being taken up from the basilar membrane. *C* hyde- $\text{OsO}_4$ , Araldite-uranyl acetate. *BM* basilar membrane. *IP* inner pillar cell. *OHC* first row outer cell. *OP* outer pillar cell. *OS* outer spiral vessel. *PS* perilymphatic space of scala tympani. *ST* stria space. *TS* tunnel space. Bars indicate 1  $\mu\text{m}$ .



panic cover layer and in the fibrocytes of the spiral limbus. On the other hand, no particles of  $\text{ThO}_2$  were seen in the pillar cells or Deiters' cells or in the fluid spaces of the tunnel in the 20-, 30-, or 40 minute experiments (Fig 3f).

In the ears fixed 2 hours or more after beginning the administration of tracer,  $\text{ThO}_2$  particles were observed in the pillar and Deiters' cells and in the tunnel spaces in all sections examined. The amount of incorporation of tracer varied greatly, however, from place to place in the cochlear spiral. Since we were interested in the fate of the material after it had entered the tunnel, we chose to look at areas with maximal concentration of  $\text{ThO}_2$  particles in the fluid spaces and supporting cells (Fig 3a-e).

Once the tracer material has entered the tunnel spaces, apparently by way of the supporting cell bases (Fig 3d, e), it is found uniformly distributed in vesicles in all parts of the supporting cells including the head plates (Fig 3a, b) and stalks (Fig 3c) of the pillar cells, and the phalangeal processes of Deiters' cells. The material is also seen densely applied to the surface coats of supporting cells (Fig 3b). We have not observed  $\text{ThO}_2$  particles either along the membranes or in the cytoplasm of the outer hair cells. It appears frequently, however, surrounding the upper tunnel crossing nerve fibers and occasionally in the axoplasm of these fibers.

#### Toxic reactions

In an effort to investigate the possibility of toxic reaction induced by the presence of Thorotrast, two additional ears were perfused with a 25% aqueous solution of potato dextrin. The appearance of cytoplasm in various cell types did not differ appreciably between animals given Thorotrast or dextrin alone, from this observation we conclude that  $\text{ThO}_2$  itself is not toxic to cells of the organ of Corti.

In untreated guinea pigs the number of clear vesicles seen in tympanic cover layer fibrocytes and pillar feet was similar to that in the experimental animals, suggesting that pinocytotic activity is not generally stimulated by the addition of the dextrin containing preparation.

It is, of course, admitted that some adverse reactions may occur due to experimental conditions such as deprivation of perilymph, cooling of the otic capsule in the exposed condition. These appear to be minimal, however, within the time periods used in our experiments and to be confined to slightly swollen mitochondria in the hair cells (visible in Fig 3).

In summary, the results of our tracer experiments indicate no passive diffusion of Thorotrast into the fluid spaces of the tunnel of Corti, none was seen there in experiments lasting 15 min or less. The amount of active transport by the supporting cells varied unsystematically region to region of the cochlea in our experiments, however, and it remains a possibility that in areas of high activity active transport could occur in a very short time. Interaction of the part of the supporting cells by pinocytosis or cytotransport (as discussed by Vosteen 1971) with perilymph near the cell bases and the fluid of the tunnel and the Nuel spaces is demonstrated.

#### DISCUSSION

There are few previous observations of a particular morphological character which would lead to, or even hint at, the idea of a fluid transport function for the pillar cells. Various published electron micrographs including the fine structure of Angelborg & Engstrom (1972) demonstrate the presence of mitochondria in adequate numbers for oxidative metabolism related to a high level of surface activity. Mitochondria are concentrated in the cytoplasm of the pillar cells (which contain the nucleus) and are also found in the stalks and heads between the terminal segments (stalks), terminal web (heads) and external membrane. The horseradish peroxidase experiments made by de Lorenzo et al (1971) led him to suspect an active role on the part of the supporting cells in the metabolism of the organ of Corti. With SEM we have observed a substantial distribution of microvilli on the external face of the pillar cells facing the tunnel (Fig 2), in addition to the openings demonstrated in this paper.

Certain histochemical enzymological findings the literature can now be interpreted in light morphological observations made with and without the benefit of exogenous tracers. Sternhagen (1970) found high concentrations of protein bound sulphydryl and disulfide groups in the stria vascularis and pillar cells of guinea pigs, as demonstrated by the DDD reagent. These concentrations were reduced in animals suffering from chronic experimental arsenic or mercury poisoning. Energy metabolism involved in transport processes would be expected to respond in this manner to heavy-metal poisoning. Enzymes associated with oxidative metabolism, including DPN and TPN-diaphorase and cytochrome oxidase, have been demonstrated on the surface of pillar cells (Gerhardt, 1962*a, b*, Lim, 1970). Nakai & Hilding (1967) observed ATPase activity on supporting cells of Corti's organ. We were surprised at the presence of this enzyme in the subreticular region. In our laboratory ATPase has been shown to be specifically associated with the pillar head plates (Thomas P. Jr, in preparation). Spoendlin & Balogh (1963) reported strong succinic dehydrogenase activity in the upper parts of the Denter's cells and remarked that this finding suggests a functional role more general than that of providing physical support for the sensory cells. Openings in pillar cell surfaces and actual channels possibly involved in fluid transport are clearly evident in our electron microscopic observations, although their apparent overall infrequency of occurrence might lead us to question their functional significance. Since it appears, however, that their formation and disappearance may be a dynamic process the small numbers seen in some specimens need not force us to discount the possibility of an important role. Further evidence available to support the idea of functional continuity between the fluid spaces on either side of the reticular lamina comes from electrophysiological measurements of Lawrence et al. (1974) who were unable to demonstrate a difference in DC potential between the tunnel and the subreticular fluids. Our failure to detect  $\text{ThO}_2$  particles in the

tunnel spaces in three animals with 20-, 30-, and 40-minute Thorotrast perfusions, makes it difficult to believe that passive diffusion actually occurs across the basilar membrane. In these animals, moreover, the material was present in great concentration in the basilar membrane to the level of the basal lamina underlying the pillar and Denter's cells. Above this level, and in the tunnel the observed concentration of Thorotrast is smaller in the majority of sections examined even in tissue samples from the long term experiments. The existence of a large concentration gradient across the membrane argues, by definition, against passive diffusion as a transport mechanism. This claim is at odds with the interpretation of von Ilberg (1968*b*) restated by Vosteen (1970) that passive diffusion between the scala tympani and the tunnel spaces occurs, and that the tunnel fluid is actually perilymph. Von Ilberg states (1968*b*, p. 390) that after 30–60 minutes  $\text{ThO}$  particles were found on the cell surfaces lining the tunnel space—a statement not incompatible with an active transport mechanism. Although the electrolyte composition of the tunnel fluid is not yet known, and may be found to resemble closely that of perilymph, our contention that active transport occurs across the feet of the pillar cells is in accord with the concept of cortilymph as proposed by Engstrom (1960) and reviewed by Engstrom et al. (1965).

We believe that the difference in apparent affinity for the tracer between hair cells and supporting cells which was consistently evident in our experiments may constitute a significant observation. It is also noteworthy that  $\text{ThO}_2$  particles were seen in high concentration opposed to the stria vascularis in cochleas in which large amounts of tracer entered the scala media. This phenomenon is now being investigated and we are attempting to determine what surface charge, if any, exists on the colloidal particles in Thorotrast which might explain the distribution of the substance over the membranes of various cell types in the organ of Corti.

As this work proceeds we hope to learn more about the special properties of the supporting cells which permit them to play an important

role in the maintenance of microhomeostasis (Hawkins, 1973) in the cochlea

## ACKNOWLEDGEMENT

We wish to thank Ana Luisa Rodriguez, James W. Bruce, and Vernon T. Maulbetsch for skillful technical assistance. Prof. Joseph E. Hawkins, Jr. generously contributed guidance for this study and critically read the manuscript. We are grateful for the use of the SEM facility under the direction of Prof. W. C. Bigelow. Mr. and Mrs. William Metcalfe of Rochester, Michigan, donated the chinchillas used in the experiments.

## ZUSAMMENFASSUNG

Es werden elektronenmikroskopische Aufnahmen gezeigt, die Öffnungen in den Kopfplatten der Pfeilerzellen des Cortischen Organs erkennen lassen. Anhand dieser Befunde wird die Möglichkeit eines Flüssigkeitstransports zwischen den Tunnelräumen und dem subkortikalen Raum erörtert. Die Fähigkeit der Pfeilerzellen, Flüssigkeit zu transportieren, wurde ausserdem mit Hilfe des Kontrastmittels Thorotrast untersucht. Thorotrast konnte in den Tunnelräumen frühestens 40 Minuten nach Einbringen in die Perilymphe nachgewiesen werden. Nach 2 Stunden wurde Thorotrast in Vesikeln in allen Teilen der Pfeilerzellen sowie an der äusseren Membran gefunden. Nach Betrachtung histochemischer Beobachtungen an Pfeilerzellen, sowie aufgrund der morphologischen Befunde und der Ergebnisse der Thorotrast-Experimente hat es den Anschein, dass diese Zellen an aktiven Transportprozessen beteiligt sind, die die Perilymphe, Cortilymphe und die Flüssigkeit des subkortikalen Raumes umfassen. Diese können dazu dienen, die Mikrohomeostase im Schneckenrezeptor aufrecht zu erhalten.

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## Na-K-ATPase ACTIVITY IN THE COCHLEA OF THE RAT DURING DEVELOPMENT

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(Received January 10, 1974)

The development of the Na-K-activated ATP-enzyme system was studied in the stria vascularis from 6-46 days post partum. In 6-8-day-old animals a rather high Na-K-ATPase activity was observed. After the activity increased gradually until the 12th day a surprisingly sharp increase of the Na-K-ATPase activity was found from 12-18 days. The adult level was reached between 19 and 25 days after birth, which coincided with the functional maturity of the cochlea. These results together with comparable data on the development of the ERP and the ionic composition of the endolymph, confirm the assumption that the Na-K-ATPase system in the stria vascularis is responsible for the maintenance of cation gradients and for the generation of the ERP.

Many studies have been performed on the morphological and functional development of the cochlea. The majority of these experiments deals with the anatomical development of the cochlea and its structures in connection with the development of the cochlear potentials.

Enggård (1965) observed in rabbits a close relationship between the development of the cochlear potentials, the morphological maturation of the cochlear tissues and the response to acoustic stimulation. In rats a relationship between the development of the electrical activity and the response to sound stimulation has been demonstrated by the experiments of Schmidt & Hernandez (1963), Crowley & Hepp-Reymond (1966) and Bosher (1972).

Although many studies have been performed on the adult cochlea on the relationship between metabolic processes in the cochlear tissues and the electrical activity (Konishi et al, 1961,

Konishi & Kelly, 1968, Bosher & Warren, 1968, Thalmann et al, 1973) up to the present, no such experiments have been performed during the ontogenesis of this organ.

In view of the important role of the intracellular-like ionic composition of the endolymph in cochlear function, we studied in earlier experiments the distribution of the Na-K-ATPase activity in the adult organ. In many tissues this enzyme system has been shown to be intimately connected or even identical with the cation pump system. The Na-K-ATPase system in the stria vascularis was concluded to maintain the cationic gradients between endolymph and perilymph or blood (Kuypers & Bonting, 1969, Konishi & Mendelsohn, 1970). Moreover a close relationship between the functioning of this enzyme system and the existence of the endocochlear resting potential (ERP) was established. In addition a strong dependency of the cochlear micro-

The present study was undertaken to trace the development of the Na-K-ATPase activity during the ontogenesis of the cochlea in order to study its relation to the development of the cochlear function.

### METHODS

The experiments were performed on young rats of an inbred strain. For dissection the

rats aged from 6–46 days (the average gestation time was 22 days) were killed by decapitation. A reliable method for the dissection of the stria vascularis in animals younger than 6 days appeared to be impossible.

After removing the lateral wall of the middle ear and the mucous substance, present in the middle ear space of the younger animals, the cochlea was exposed. Subsequently the bony capsule of the cochlea was carefully removed. The spiral ligament with stria vascularis of the lower two turns was detached from the modiolus and the stria vascularis dissected and lyophilized as described before (Kuypers & Bonting, 1969).

ATPase activities were determined in homogenates of the stria vascularis (4 specimens for each assay). The methods used have been described earlier (Kuypers & Bonting, 1969).

For histological studies the animals were perfused by intravitam perfusion with formaldehyde and, after decalcification, embedded in paraffin and sectioned.

## RESULTS

In preliminary experiments ATPase activities were determined in the tissue complex consisting of stria vascularis and spiral ligament. However reproducible data could be obtained. This failure was partly due to a variable amount of bone chips adhering to the spiral ligament, which

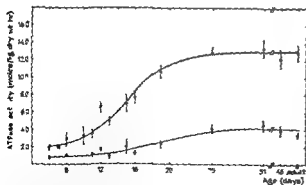


Fig. 1 Mg (●) and Na-K (○) activated ATPase in stria vascularis homogenates from rats at different ages. Each point represents the mean value with S.E. of 4 determinations (MKH: moles ATP hydrolyzed/kg dry weight/hr at 37°C).

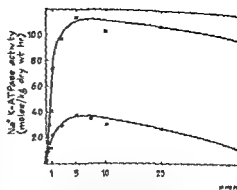


Fig. 2 Effect of various  $K^+$ -concentrations on the Na-K-ATPase activity in the presence of 60 mM  $Na^+$  and 25 days post partum.

could not be removed in an adequate way. Therefore the enzyme activities were measured on isolated stria vascularis.

Fig. 1 shows the Na-K- and Mg-activated ATPase activities in the stria vascularis at different ages. In the 6- and 8-day old animal already a high amount of Na-K-ATPase was found (2.5 MKH). During the days this activity gradually increased. However from 12–18 days after birth a very rapid increase of the Na-K-ATPase activity of the stria vascularis occurred, from 4–10 MKH. The addition of both Na-K- and Mg-activated ATPase activity was reached between 19 and 25 days post partum. Histological examination of the cochlear structures at that age showed a more anatomical structure.

Figs. 2 and 3 show the activation by  $K^+$  of the Na-K-ATPase system at 10 and 25

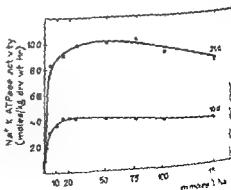


Fig. 3 Effect of various  $Na^+$ -concentrations on the Na-K-ATPase activity in the presence of 5 mM  $K^+$  and 25 days post partum.

Table I Activities and properties of ATPase in cochlea of various animals

	Guinea pig stria vasc.	Chicken tegg. vasc.	Rat stria vasc.
ATPase activity (H)	7.0	8.0	13.0
ATPase activity (H)	5.6	12.4	3.8
maximal Na <sup>+</sup> -conc	4.5	4.9	2.0
maximal K <sup>+</sup> -conc	0.9	0.9	1.0

μ moles ATP hydrolyzed (kg dry weight) hr<sup>-1</sup> at 37°C

birth. At both ages half maximal activation reached at 1.0 mM K<sup>+</sup> in the presence of 60 mM Na<sup>+</sup> and at 2 mM Na<sup>+</sup> in the presence of 10 mM K<sup>+</sup>. Maximal activation was obtained at 30 mM Na<sup>+</sup> and 5 mM K<sup>+</sup>.

## DISCUSSION

The data presented in Fig. 1 show that the ATPase activities in the stria vascularis of the rat reach the adult level between 19 and 25 days after birth. These data are in good agreement with the morphological maturation of the cochlear structures, the maturity of the electrical potentials and the response to sound stimulation (Hamdani & Fernandez, 1963; Crowley & Heppner, 1966; Boshier, 1972).

In Table I the data obtained on the ATPase activities in the full grown cochlea of the rat are presented together with identical data obtained in the guinea pig stria vascularis (Kuypers & Bonting, 1969) and the tegmentum vasculosum (homologous to the stria vascularis) in the chicken for purposes of comparison (Kuypers & Bonting, 1970). These structures have in common extremely high Na-K ATPase activity compared with other tissues in which cation transport occurs against an electrochemical gradient (Bonting, 1970). These findings in the rat give additional support for our assumption that this enzyme system in the stria vascularis and the tegmentum vasculosum plays a central role in

the maintenance of the cationic gradients between the endolymph and the perilymph or blood.

From the enzyme data obtained during the ontogenesis of the rat cochlear structures (Fig. 1) it appears that the stria vascularis already at 6-8 days post partum showed a considerable amount of enzyme activity (2.5 MKH). Boshier (1972) demonstrated in 8-day old rats a nearly adult ionic composition of the endolymph, while the ERP was only a few mV. In view of these findings it seems likely to assume that this amount of enzyme activity is able to maintain the intracellular like ionic composition of the endolymph at that age. The further increase of the Na-K-ATPase activity from 4.0-10.0 MKH, observed between 12 and 18 days seems not to be essential for the maintenance of the cationic gradients, since no measurable increase in the size of the endolymphatic space was observed during this period. In about the same period, between 12 and 15 days, Boshier (1972) observed a very sharp rise of the ERP from -20 to -85 mV, without significant changes in the ionic composition of the endolymph. From earlier experiments a close connection between the functioning of the Na-K ATPase system in the stria vascularis and the existence of the ERP was established (Kuypers & Bonting, 1970). The nearly simultaneous increase of the Na-K-ATPase activity and the ERP is in agreement with this assumption. According to this hypothesis an increase of the ERP implicates that more K<sup>+</sup> ions had to be pumped into the endolymph, since the Nernst potential for K<sup>+</sup>-distribution (figures for K<sup>+</sup>-conc. derived from Boshier, 1972) is -80 mV, a greater deviation from the Nernst potential requires more energy, i.e. an increase of the ATPase activity (Nernst potential  $E = -(RT/F) \ln(K^+_{\text{endol}}/K^+_{\text{peril}})$  mV). Since no difference in the activation curves by Na<sup>+</sup> and K<sup>+</sup> are observed (Figs. 2 and 3) before and after the sharp rise of the ERP it seems unlikely that this rise can be attributed to a change in the characteristics of the enzyme system. However, a contribution to this rise of the ERP by changes in the resistance of the membranes of the cochlear



duct as suggested by Bosher (1972) cannot be completely excluded

In conclusion it can be stated that these results confirm our former assumption that the Na-K-ATPase system in the stria vascularis is responsible for the maintenance of the cationic gradients between endolymph and perilymph or blood and for the generation of the ERP

## ACKNOWLEDGEMENTS

The author expresses his gratitude for the excellent technical assistance of Miss D. P. C. Wilberts

## ZUSAMMENFASSUNG

Die Entwicklung des Na K aktivierten ATPase Systems wurde in der Stria vascularis von 6 bis 46 Tage alten Ratten untersucht. Bei 3 bis 8 Tage alten Tieren wurde bereits eine ziemlich hohe Enzymaktivität gefunden. Nach einer langsamen Zunahme bis zum 12. Tag wurde eine sehr grosse Steigerung bis zum 18. Tag observiert. Das erwachsene Niveau wurde zwischen 19 und 25 Tagen post partum erreicht. In derselben Zeit ist auch die Schnecke funktionell ausgewachsen. Diese Ergebnisse zusammen mit vergleichbaren Daten zum Verhalten des ERP und des Ionengehalts der Endolymphe, stützen die Auffassung, dass das Na K ATPase System in der Stria vascularis für die Aufrechterhaltung der Kationengradienten und für die Erzeugung des ERP, verantwortlich ist.

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## ADRENERGIC INNERVATION IN THE RABBIT COCHLEA

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(Received February 15, 1974)

**Abstract.** The distribution of adrenergic nerve fibres in rabbit cochlea was investigated with fluorescence and on microscopic techniques. Interest was focused on the stria vascularis. After administration of a false transmitter, the fine structure of adrenergic nerve fibres and their relationship to other structures were studied. A large number of adrenergic nerve terminals were found in the stria vascularis, radiating nerve fibres where they lose their myelin sheaths. Apart from this nerve fibre innervation, there was an extensive innervation of the spiral vessel of the cochlea, lying under the inner hair cell. This blood vessel was of capillary size and lacked contractile elements. No adrenergic innervation was observed in the organ of Corti or in the stria vascularis. The cochlear adrenergic innervation was found to have its origin in the superior cervical ganglion. Possible ways of adrenergic influence on sound perception were discussed.

Adrenergic innervation of the inner ear has attracted much attention since it was suggested that several diseases of the inner ear, such as Meniere's disease, vertigo, and sudden deafness, may be due to an autonomic imbalance (Seymour & Spoor, 1951, 1953; Pásse, 1953). The influence of sound perception and blood circulation in the inner ear have been investigated under various experimental conditions. A sympathetic effect on cochlear microphonics has been registered, but the results have not been clearcut (Beickert et al., 1956; Rambo et al., 1953; Seymour & Spoor, 1951). The morphology of the vascular system in the cochlea has also been studied but the function has been difficult to assess in relation to the endo- and perilymphatic fluids.

This work was supported by grant no. 04x 2461 from the Swedish Medical Research Council and a grant from Karolinska Institute.

and the supply of the organ of Corti with oxygen and nutrients.

Injuries to the stria vascularis do not seem to affect the organ of Corti, while obstruction of the spiral vessels causes a degeneration of the hair cells (Lawrence, 1966; Alford et al., 1965). Blood vessels in the stria vascularis and the spiral vessels have been observed during stimulation of the cervical sympathetic trunk. No visible changes were seen in flow rate or diameter of these vessels (Perlman & Kimura, 1955).

As a basis for an investigation of the adrenergic influence in the cochlea, the morphology of the adrenergic innervation has first to be considered. Earlier histological methods for light microscopy, such as silver staining, were non specific and coloured all non myelinated nerve fibres and even connective tissue (Palumbi, 1954; Andrzejewski, 1955, 1956). Thus it was difficult to clearly distinguish adrenergic nerve fibres from other fibres. By means of the fluorescence method specific for catecholamines (Falck et al., 1962), cochleas from cats (Spoendlin & Lichtensteiger, 1966, 1967), rabbits, rats and guinea pig (Vinnikov et al., 1966) and guinea pigs (Terayama et al., 1965, 1966) have been investigated. Vinnikov et al. (1966) reported the existence of adrenergic nerve fibres in the inner spiral bundle, but apart from this, no adrenergic innervation was to be found in the organ of Corti or stria vascularis. The cochlea is difficult to investigate

because it is embedded in bone, and bony lamellae surround the spiral ganglion and nerve fibres radiating to the organ of Corti. Terayama et al (1965, 1966) used cryostat sectioning and they claimed the innervation to be a pure blood vessel innervation ending at the small vessel under the inner pillar or the tunnel of the organ of Corti. However, subsequent electron microscopic studies failed to demonstrate adrenergic nerve terminals around the spiral vessels beneath the inner pillar and tunnel or at the habenula perforata (Terayama et al, 1968). When specimens were treated according to the Champy-Maillet zinc iodide-osmic acid technique (Hawkins, 1968), light microscopy demonstrated "a vasomotor innervation by unmyelinated nerve fibres that accompany the myelinated cochlear fibres but descend to pierce the basilar membrane and terminate on the vessels" under the tunnel of Corti. In stretch preparations, Spoendlin & Lichtensteiger (1966, 1967) found an innervation of blood vessels in the modiolus and an arcade-like habenular plexus independent of blood vessels. No definite adrenergic innervation has been found distal to the inner hair cell.

In an electron microscopic study on cats, where a false transmitter was administered, continuous blood vessel innervation was found extending as far as the spiral vessel of the tympanic lip. A blood vessel independent plexus was found around nerve bundles in the habenula region and especially around the non myelinated part of the axon (Densert & Flock, 1974).

There are marked anatomical differences between cat and rabbit concerning the internal carotid artery, which is lacking or rudimentary in cat. This is probably the reason why all sympathetic nerve fibres to the head pass the middle ear in cat.

Because of its species differences, and since the rabbit is well suited for experiments in cochlear physiology it warrants a separate description. The aim of the present study was to investigate the adrenergic innervation of the rabbit cochlea by means of fluorescence and electron microscopic techniques and by nerve

sectioning, to try to find the sympathetic pathways to the inner ear.

## MATERIAL AND METHODS

In this study 19 white rabbits weighing from 12 to 4.0 kg were used.

### Fluorescence microscopy

Five normal rabbits were used. In order to enhance the fluorescence, L-dopa (10 or 20 mg/kg i.p.) was administered to some animals one hour before decapitation. In order to deplete the monoamine stores, reserpine (10 mg/kg i.p.) was administered 24 hours before sacrifice. In 2 animals the tympanic bulla was opened on one side and the tympanic plexus was cut. This was done to evaluate the proportion of sympathetic fibres passing to the inner ear via the tympanic plexus. Unilateral cervical sympathectomy was performed in 2 animals. Operations were performed about 3 weeks before sacrificing. Animals were decapitated under Nembutal® anesthesia and the cochleas were dissected out as quickly as possible. They were freeze-dried and then treated with formaldehyde gas according to the histochemical fluorescence method of Falck & Hillarp (1962), (for details see Falck & Owman, 1965 and Corrodi & Jonsson 1967). Instead of embedding in paraffin, the specimens were infiltrated *in vacuo* for about 15 minutes in Epon, preheated to 60°C. The epoxy resin was hardened and serial sections of segments of the cochlea were cut on a Jung microtome with glass knives.

The sections were generally 6 to 8 µm thick and mounted in Entellan®. A Zeiss fluorescence microscope with a dark field condenser was used. In this microscope, fluorescent products of catecholamines appear green to yellow green in colour and those from 5-HT are yellow. Photomicrographs were taken using Scopix G film.

### Electron microscopy

Six rabbits were pretreated with 5-OH DA (3,4,5-trihydroxyphenylethylamine hydrochloride) 4 × 20 mg/kg i.v. over a period of 48 hours (Tranzer & Thoenen, 1967).

### Pre sectioning

one animal unilateral sectioning of the vagus plexus was performed. After 3 weeks an animal was pretreated with 5 OH DA and reduced.

Unilateral sympathectomy was performed in animals. One of these had a survival time of 3 weeks and was pretreated with 5 OH DA while second animal was sacrificed after 8 days and was not pretreated with 5 OH DA.

The animals were sacrificed by decapitation after Nembutal<sup>®</sup> anesthesia. The cochleas were fixed in one of two fixatives:

(a) 3% glutaraldehyde (0.133 M sodium phosphate buffer pH 7.3) followed by 1% osmium tetroxide buffered in veronal acetate (Rhodin 1954) for 2 hours.

(b) Ice-cold 3% potassium permanganate in 0.1 M sodium phosphate buffer at pH 7.0 for 10 minutes (Richardson 1966; Hokfelt 1968; Hokfelt & Jonsson 1968). After rinsing in Ringer solution the specimens were contrast stained en bloc in ice-cold 1% uranyl acetate in Ringer solution for 60 minutes.

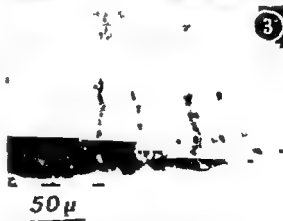
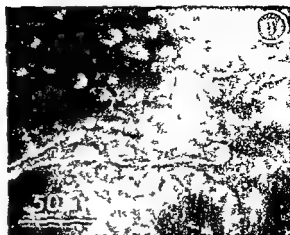
The fixed specimens were dehydrated in ethanol and embedded in Epon (Luft 1961). Ultrathin sections were cut with glass or diamond knives on a LKB ultratome. Diamond knives were preferred because the bony spirals which enclosed the radiating nerve fibres were included in the specimens. Sections from specimens fixed in potassium permanganate were contrast stained only briefly in lead citrate (Reynolds 1963) while osmium fixed sections were contrast stained in uranyl acetate (Watson 1958) and lead citrate. The sections were examined in a Siemens Elmiskop I electron microscope.

## RESULTS

### Fluorescence microscopy

1 catecholamine-containing nerve fibres fluoresce green as an indication of the presence of adrenaline. In the cochlea no cell bodies were seen to contain biogenic amines.

In the modiolus there was a moderate number of adrenergic nerve fibres mostly coursing along



vestibular ganglion

varicosities  
of the  
cochlea  
ion 325

of the tympanic  
nerve. From the  
<375

second turn, 6 μm thick p.p. as seen in <375

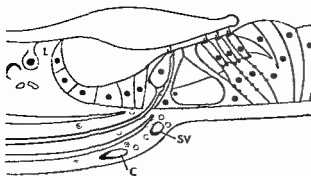


Fig. 4. Schematic diagram illustrating the adrenergic innervation in the rabbit cochlea. SV, spiral vessel of the tympanic lip, C, radiating capillary, L, limbus, O, adrenergic nerve terminal.

the longitudinal axis of the modiolus. There seemed to be a moderate, continuous, adrenergic perivascular innervation. The plexus cochlearis (Balogh & Koburg, 1965) was included in some sections, but no concentration of adrenergic fibres was seen in this region of the modiolus. Adrenergic fibres did not seem to have any relationship to blood vessels.

In the spiral ganglion, adrenergic nerve fibres were mostly seen to pass through the ganglion, sometimes together with blood vessels. Only occasionally were adrenergic nerve fibres seen to lie close to or partly encircle a ganglion cell with shining varicosities close to the cell wall (Fig. 1).

The ganglion cells frequently contained yellow-red autofluorescent particles in the cytoplasm.

In the rabbit the osseous laminae around the radiating nerve fibres were thick until they ended at the level of the habenula openings. In stretch preparations it was therefore difficult to distinctly identify and separate adrenergic nerve fibres.

In plastic sections, adrenergic nerve fibres were seen to run along the radiating nerve bundles, mostly as separate nerve fibres with varicosities. It was difficult to ascertain whether adrenergic nerve fibres followed blood vessels or ran independently.

In the habenula region, however, one could often distinguish blood vessel innervation and innervation independent of blood vessels. Blood

vessel independent adrenergic nerve fibres were seen close to radiating nerve bundles, a concentration of varicosities just before habenula openings (Fig. 2). An intense fluorescence was also seen in association with the vessel of the tympanic lip (Fig. 3). In the cochlear turns were investigated that the most extensive innervation in the habenula region existed in the second turn. The spiral vessel seemed to be broad, intense shining bands and single varicosities could not be resolved. In sections from the limbus region an adrenergic innervation was seen. Some fibres were seen to follow blood vessels more often. Nerve fibres encircled cell bodies of an autofluorescent material.

In the organ of Corti and stria vascularis catecholamine fluorescence was detected in stretch preparations or in sections.

After unilateral cervical sympathectomy fluorescence disappeared in the cochlea operated side.

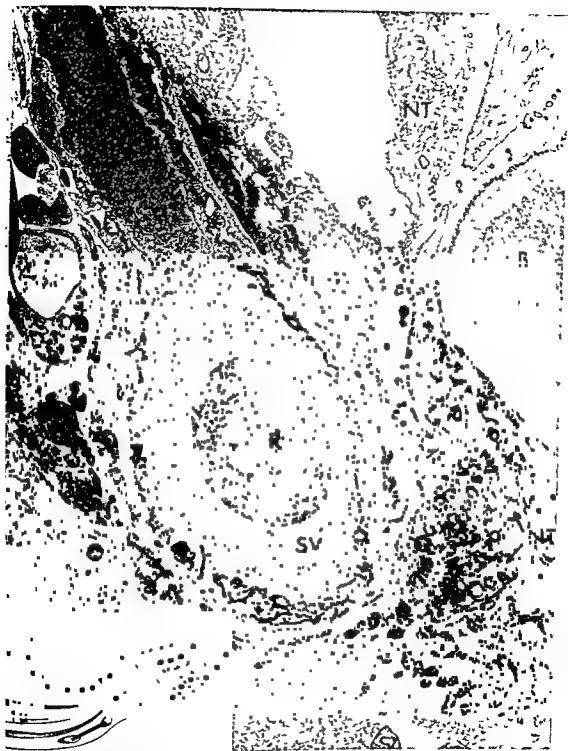
Sectioning of the tympanic plexus did not influence the innervation pattern in the cochlea.

After administration of reserpine all catecholamine fluorescence disappeared.

### Electron microscopy

After administration of the false transmitter 5-OH-DA, adrenergic nerve terminals were easily recognized and differentiated from other types of terminals. Adrenergic synaptic vesicles contained a precipitate consisting of the reaction product between the amine complex including 5-OH-DA and the fixative (Tranzer & Thoenen, 1967; Hökfelt & Jonsson, 1968; Hökfelt, 1968). Potassium permanganate fixed terminals were recognized by the fact that these vesicles contained a dense core. The vesicles were about 1000 Å in diameter but some were larger. Mitochondria were generally present near the terminals.

The cochlea had a very rich adrenergic innervation, with a concentration to the habenula region. With the electron microscopic technique used it was possible to distinguish clearly



5 Electron microscopic section from the cochlea are a radiating capillary (RC) joins the spiral vessel the tympanic lip (SV). Groups of adrenergic nerve

terminals (NT) are seen close to the blood vessel B, bone, R, red blood corpuscle. Fixation in potassium permanganate  $\times 12\,600$



Fig. 6. Part of the spiral vessel of the tympanic lip (SV) and groups of adrenergic nerve terminals (NT). Fixation in potassium permanganate:  $\times 30,750$ .

innervation associated with blood vessels: afferent nerve fibres and free terminals in the connective tissue.

#### Blood vessel innervation

In the rabbit the outer spiral vessel was absent. The spiral vessel of the tympanic lip (Fig. 4) was

built up of capillaries. The osseous spiral lamina encloses the spiral vessel in the tympanic lip region and the

travers



7 Electron microscopical section from the habenula  
 A row of adrenergic nerve terminals (NT) along

a myelinated nerve fibre (NF) where it loses its myelin sheath. Fixation in potassium permanganate.  $\times 24,300$

The arterial blood vessels in the spiral lamina and the tympanic lip were of capillary type. They were built up of endothelial cells and erythrocytes but lacked a surrounding layer of smooth muscle cells.

In sections from the spiral lamina adrenergic nerve terminals were found either singly or in groups along the capillaries. The terminals were either partly surrounded by the Schwann cell or the radiating capillaries joined the spiral lamina and one or two distinct groups of adrenergic nerve terminals were regularly found and in some sections each group might contain up to ten terminals. These groups of terminals did not seem to be surrounded by a Schwann cell, but they were free and in other places the whole group was partly enfolded by a distended Schwann cell protrusion and it was not possible to decide whether it originated from a Schwann cell or a fibroblast. The terminals faced the endothelial cells

of the blood vessel (Fig. 5). The bony lamellae of the spiral lamina could extend beyond the spiral vessel which was sometimes enclosed by bone in three directions. Adrenergic nerve fibres had a typical appearance with regular swellings, i.e. terminals filled with vesicles. Along the spiral vessel however this arrangement was partly concealed by many terminals assembled in elongated clusters. In one ultrathin section as many as fifty terminals could be counted in a cluster. Sometimes the spiral vessel was totally surrounded by terminals. The existence of Schwann cells around the terminals was not a regular finding. In some places a bundle of adrenergic axons might be surrounded by Schwann cells but mostly no Schwann cell was observed. The nature of observed elongated cell protrusions was not possible to decide (Fig. 6).

In the apical part of the cochlea the blood vessel innervation was less pronounced. Single



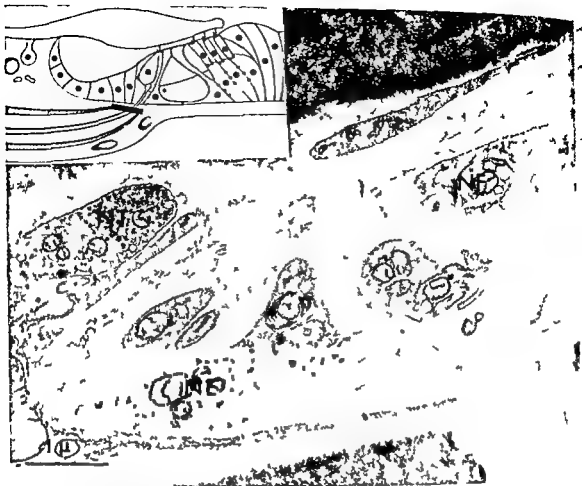


Fig. 8. Electron microscopical section from the habenula region illustrating the relationship between nerve fibres (NF) after they have lost their myelin sheaths and an

adrenergic nerve terminal (NT). Fixation in potassium permanganate.  $\times 20\,000$ .

terminals were seen along radiating capillaries in the spiral lamina but the pronounced innervation was not seen where these capillaries joined the spiral vessel. Along the spiral vessel there were rows of terminals partly covered by a Schwann cell.

#### *Afferent nerve fibre associated innervation*

Apart from the blood vessel innervation there was also an innervation associated with afferent nerve fibres. Along radiating afferent nerve bundles and between myelinated nerve fibres rows of adrenergic nerve terminals were found. The number of terminals increased towards the habenula region where terminals were located in rows or in groups (Fig. 7). Groups of terminals

were frequently seen around the afferent nerve bundles where the axons lose their myelin sheaths and turn upwards. In horizontal sections adrenergic nerve terminals were seen around the nerve bundles after they lost their myelin sheaths in the habenula (Fig. 8). The nerve bundles were surrounded by flat fibroblasts and the adrenergic nerve terminal always seemed to be located outside these cells. It was observed that adrenergic terminals and groups of terminals mostly were devoid of a Schwann cell.

#### *Free terminals*

In the connective tissue towards the scala tympani and scala vestibuli terminals were

and without any connection to blood vessels or nerve fibres. These nerve terminals were usually devoid of a Schwann cell cover. Nerve terminals were more abundant in the tissue around the scala tympani, especially in the neighbourhood of the spiral vessel.

#### Free sectioning

When the tympanic plexus was resected there was no definite change in the cochlea with regard to the amount or arrangement of nerve terminals.

After cervical sympathectomy all adrenergic nerve terminals disappeared completely. After resection of the area around the spiral vessel of the tympanic lip, where the innervation was most abundant, appeared empty. Even after 8 days the nerve terminals were degenerated and it was not possible to identify any remaining terminals.

No adrenergic innervation existed distal to the habenula region, in the organ of Corti or in the stria vascularis.

## DISCUSSION

Falck-Hillarp histochemical fluorescence method is highly specific for the demonstration of certain biogenic amines (Falck et al., 1962, 1966; Jonsson, 1967). This method has previously been used to show the presence of adrenaline-containing nerve fibres in cat, guinea pig and rabbit cochlea (Sjoendlin & Lichtensteiger, 1966, 1967; Terayama et al., 1966; Vinnikov et al., 1966). In the present study plastic embedding was used in preference to stretch preparation or cryostat sectioning, because in this way it is possible to cut parts of the cochlea and section them at different thicknesses and angles. Fluorescent nerve fibres were not masked by the yellow autofluorescence of overlying bony lamellae. Thus continuous adrenergic innervation was found in distinct varicosities along nerve bundles at the level of the habenula openings. As in cat and guinea pig (Sjoendlin & Lichtensteiger, 1966, 1967; Terayama et al., 1965, 1966), it was noted that there is a concentration of

adrenergic innervation to the habenula region, encircling myelinated nerve bundles where the axons lose their myelin sheaths. The most intense fluorescence, however, was seen around the spiral vessel of the tympanic lip, under the habenula, where thick bands of catecholamine fluorescence were seen.

There was a close correlation between fluorescence and electron microscopic findings. Administration of the false transmitter 5 OH DA makes it possible to use the electron microscope to identify and exactly localize biogenic amines in central, as well as in peripheral, neurons. The existence of a blood-brain barrier prevents systematic administration of 5 OH DA when central monoamines are investigated. However, in the inner ear the adrenergic innervation emanates from the ipsilateral superior cervical ganglion and systemic administration of the false transmitter does lead to an accumulation in adrenergic synaptic vesicles.

Fixation in potassium permanganate (Richardson, 1966; Hökfelt, 1968; Hökfelt & Jonsson, 1968) was preferred to glutaraldehyde and osmium tetroxide (Tranzer & Thoenen, 1967), for the purpose of illustrating the ultrastructure of adrenergic nerve terminals. The existence of noradrenalin in small dense-cored vesicles, located in sympathetic nerve endings, is well documented (De Robertis & Pellegrino de Iraldi, 1961; Richardson, 1962, 1966; Hökfelt, 1968; Hökfelt & Jonsson, 1968). Accumulation of biogenic monoamines and the false transmitters in adrenergic nerve terminals was first demonstrated in electron microscopic studies by Tranzer & Thoenen (1967). Using stretch preparations, Sjoendlin & Lichtensteiger (1966, 1967) suggested a blood vessel innervation in the modiolus as well as an innervation independent of blood vessels in the periphery, giving rise to a nerve-associated habenula plexus. Terayama et al. (1965, 1966), using cryostat sectioning, claimed only a pure blood vessel innervation. In an electron microscopic study Terayama et al. (1968) failed to confirm their fluorescence microscopic finding that the spiral vessel under the inner pillar and tunnel of Corti had an adrenergic

innervation. However, the electron microscopic results obtained after administration of a false transmitter (Densert & Flock, 1974) did show a continuous blood vessel innervation of the spiral vessel of the tympanic lip and a nerve fibre associated plexus in the habenula region. In the rabbit this innervation is much more pronounced, with large groups or clusters of terminals where only single nerve terminals were found in the cat. In rabbit it was observed that most terminals around blood vessels, afferent nerve fibres, and freely in the connective tissue, were not enfolded by a Schwann cell.

In agreement with the results of Spoendlin & Lichtensteiger (1966, 1967) and Terayama et al (1965, 1966) no catecholamine fluorescence was found in the organ of Corti or stria vascularis. Guinea pigs frequently have an outer spiral vessel, under the tunnel of Corti. Using light microscopy Hawkins (1968) found an autonomic innervation of this vessel and Terayama et al (1967) found fluorescence at the site. It should be noted that the outer spiral vessel is absent in the upper turns of the cat cochlea (Smith, 1954) and it is entirely absent in the rabbit cochlea.

The possible functional significance of the adrenergic innervation in the cochlea has to be considered. It might influence (a) blood vessels, (b) hair cell metabolism, (c) afferent and efferent axons.

Different areas of the cochlea are involved in ion exchange and supply of oxygen and nutrients. The relationship between the organ of Corti and the stria vascularis is not quite clear (Kimura, 1973). Experiments on cochlear blood flow have shown that obstruction of the radial vessels from the modiolus causes a loss of hair cells in the corresponding region of the organ of Corti (Alford et al., 1965, Lawrence, 1966). This might indicate that the oxygen and nutrient supply of the hair cells comes principally from the spiral vessels (Kimura, 1973). These capillaries are composed of endothelial cells and pericytes and are devoid of contractile elements. The question arises why these vessels should be supplied with such a heavy adrenergic innervation. Apart from a scanty innervation of capil-

aries in the limbus, other capillary areas in the cochlea lack an adrenergic innervation. Perlman & Kimura (1955) observed that sympathetic stimulation did not seem to influence the blood flow in the stria vascularis. Noradrenaline might influence the permeability of the spiral vessel. However, there is a very large number of terminals in this area and it does not seem reasonable to believe that influence on permeability is the only physiological function of this heavy innervation. One possibility, that must be considered, is that catecholamines may have a direct influence on the hair cell metabolism. A complex relationship exists between noradrenaline, cyclic AMP, and ions such as  $Ca^{++}$  and  $K^{+}$ . The mode of action of noradrenaline depends on whether the receptors are of  $\alpha$  or  $\beta$  type and in the inner ear this is unknown.

Only a few investigations have been carried out on the sympathetic influence on cochlear microphonics and the results are contradictory. Beickert et al (1956) reported that the sympathetic influence on cochlear microphonics appeared when the tendons of the middle ear muscles were cut. Seymour & Tappin (1964) observed an initial small rise in cochlear microphonics, followed by a decrease in amplitude.

Except for a possible direct effect on hair cells, there is morphological evidence for sympathetic influence even in the habenula region, where afferent axons have lost their myelin sheaths. Just below the basilar membrane the nerve bundles are surrounded by adrenergic nerve terminals and prominent groups of nerve terminals are regularly found around the nerve bundles. This is the site where the action potentials are supposed to be generated. Noradrenaline released in this area might influence the action potential hold for nerve impulse initiation.

## ACKNOWLEDGEMENTS

The author is deeply indebted to Ass. Prof. Åke Flock and Prof. Jan Wersäll for their support and financial aid. The author wishes to express his appreciation to Marie Louise Spångberg and Mrs. Britta Flock for all technical assistance and especially to Mrs. Britta Flock for artistic drawings.

## ZUSAMMENFASSUNG

Die Verteilung der adrenergen Nervenfasern in der Membranocochlea wurde mit Fluoreszenz- und elektronenmikroskopischen Methoden untersucht. Das Interesse vor allem der Habenuläre Region. Nach Verabreichung des falschen Transmitters wurde die Feinstruktur der adrenergen Nervenfasern und ihre Beziehung zu anderen Strukturen studiert.

Eine große Anzahl von adrenergen Nervenendigungen liegt um die strahlenförmig sich ausbreitenden Nervenfasern nach Verlust ihrer Myelinscheiden beobachtet. Neben dieser Nervenfaserninnervation fand sich eine ausgedehnte Innervation des spiralen Blutgefäßes des Labyrinths unter den inneren Haaren. Dieses Blutgefäß von der Größe einer Kapillare mit kontraktile Elemente verläuft im Corti'schen Organ und in der Stria vascularis wurde keine adrenerge Innervation gefunden. Die adrenerge Innervation der Cochlea hat ihren Ursprung in dem ipsilateralen Ganglion nodosum. Die Möglichkeiten eines adrenergen Einflusses auf die Schallperzeption werden diskutiert.

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## THE ACOUSTIC IMPEDANCE CHANGE AS A MEASURE OF STAPEDIUS MUSCLE ACTIVITY IN MAN

*A Methodological Study with Elektromyography*

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(Received February 19, 1974)

The impedance change of the ear in response to sound, widely used in clinical diagnosis, is generally considered to be caused by contraction of the stapedius muscle. In the present study acoustic impedance change at 220 Hz and EMG of the stapedius muscle were compared in subjects with unilateral ear drum perforation. One per cent of the maximum impedance change was found at a sound level 5-6 dB above the one that gave half of the maximum integrated EMG amplitude. Impedance change and integrated EMG were both approximately linear functions of stimulus sound level at least 10 dB above reflex threshold. It was concluded that the impedance change gives an adequate measure of stapedius muscle activity both at threshold and suprathreshold levels. The value of suprathreshold impedance change measurements in clinical diagnosis was pointed out.

Measurement of changes in the ear's acoustic impedance in response to sound stimulation has become an important diagnostic tool (Metz, 1952; Klockhoff, 1961; Anderson, 1969; Petersen & Liden, 1972). Such impedance changes are as a rule caused only by the contraction of the stapedius muscle. The other muscle of the middle ear, the tensor tympani muscle, is activated in man only as part of a general startle reaction or in a few cases as an acoustic reflex (Liden et al., 1970). Its threshold is in any case considerably above the stapedius reflex threshold.

This study was supported by Grants from the Swedish Medical Research Council, the Medical Faculty, University of Umeå, P. Frénckners fond, B. Fromms fond and Karolinska Institutets fond.

The electromyographic activity upon contralateral sound stimulation has been recorded from the stapedius muscle in man through a perforation of the ear drum (Perlman & Case, 1939) and during operations in the middle ear under local anesthesia by Fisch & v. Schulthess (1963) and Djupesland (1965). These studies mainly concerned the latency of stapedius reflex but Fisch & v. Schulthess (1963) also studied the reflex threshold and observed that the EMG activity was dependent on the stimulus sound intensity. In none of these studies was the EMG activity correlated to the change in acoustic impedance.

In experiments on lightly anesthetized rabbits it has been shown (Borg, 1972) by the bilateral recording method developed by Møller (1961) that changes in the ear's acoustic impedance are closely correlated to the threshold for EMG activity of the stapedius muscle. Furthermore, good correlation was found between the amplitude of the integrated EMG and the change in impedance.

There is very incomplete knowledge of the correlation in man between the change in acoustic impedance during sound stimulation and the activity of the stapedius muscle as measured more directly. Jepsen (1955) compared threshold for impedance change with threshold for directly observed movement of the stapedius tendon upon contralateral sound stimulation in two

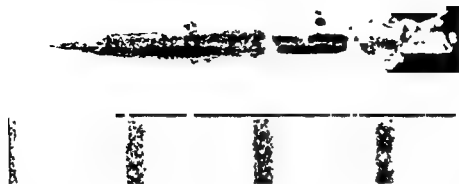


Fig 1 Tungsten electrode and millimetre scale.

patients with unilateral perforation of the ear drum. The threshold determined by direct observation was less than 10 dB higher than the threshold for impedance change.

The aim of the present work was to determine the correlation between the EMG activity of the stapedius muscle and the acoustic impedance change with respect to threshold sensitivity and suprathreshold response amplitude. This was accomplished by simultaneous recordings of ipsilateral impedance change in one ear and EMG in the other ear in patients with unilateral ear drum perforation. The results showed a good correlation between recordings obtained with the two methods.

## MATERIAL AND METHODS

The experiments were performed on a total of 37 subjects. Thirty-two of these comprised a control group previously described by Borg & Zakrisson (1974). Selected from young university students, they were used to determine the normal difference in sensitivity between the ipsilateral and contralateral stapedius reflex. Five subjects, the Experimental Group, had an ear drum perforation with visible stapes and stapedius muscle tendon in one ear. In this ear they had hearing thresholds of 20–75 dB HL (Hearing Level) according to ISO Standard (1964) in the frequency range 0.125–2.0 kHz. In the other ear they had hearing thresholds within 15 dB according to ISO Standard (1964) at

the frequencies used to elicit the stapedius reflex. Four of the subjects had normal ear drums in this ear according to otomicroscopic observation and the fifth an ear drum scar. The Experimental Group was used for EMG recordings.

**Sound stimulation** The reflex activity, both EMG and impedance change, was obtained in response to bursts of pure tone at 0.5 or 2.0 kHz (rise-time to 90% and fall time to 10% were 1 or 2 msec) of 1 sec duration. The stimulus was increased in 4 dB steps from below reflex threshold to about 130 dB SPL (sound pressure level) and then lowered again to below reflex threshold. Two stimulus presentations were thus made at each sound level.

**Impedance change** was measured with the method developed by Møller (1960, 1961). This technique allows a simultaneous recording of ipsi- and contra-lateral impedance change at 80 Hz. The 800 Hz signal representing the change of impedance during stapedius contraction was recorded on a two-channel tape recorder (Reo A 77). During playback the signal was rectified and low-pass filtered (bandwidth 32 Hz, 18 dB per octave). The amplitude of the impedance change signal was measured at the end of the stimulus and expressed in per cent of the maximum response obtained at each session. The value was plotted as a function of stimulus intensity to produce stimulus-response curves. In the control group bilateral recordings of impedance change were made. In 4 of the patients of the Experimental Group impedance change was re-

ended simultaneously with the EMG. In the first patient no impedance change was obtained which could be due to middle ear pathology related to the ear drum scar (Klockhoff, 1961).

EMG recordings were made with tungsten microelectrodes (Fig. 1) of the type described by Vallbo & Hagbarth (1968). The electrodes were coated with Araldite except for 0.4 mm at the tip. Their length was about 3 mm. The tip diameter was between 1 and 10  $\mu\text{m}$  and the impedance was about 40 kOhms at 10 kHz.

The recording electrode was introduced through the perforation of the ear drum and inserted into the tendon of the stapedius muscle. It was positioned as near the muscle as possible, parallel to the tendon. No local anesthesia or sedation of the patient was utilized. The reference electrode was a similar, but longer tungsten needle having a greater uncoated area. It was inserted into the ipsilateral ear lobe. The EMG signal picked up by the electrode was amplified by a Tektronix 2A61 amplifier with an input impedance of  $2 \times 10^6$  Ohms. The frequency response was between 60 Hz and 6 kHz which gave the best signal-to-noise ratio in these recordings. The amplified signals were displayed on a Tektronix 564 oscilloscope and monitored on a loud speaker. The activity was recorded on one channel of the tape recorder, the impedance change of the other ear being simultaneously recorded on the other channel. The integrated EMG responses were obtained by low-pass filtering the rectified EMG signal. Active low-pass filter of third degree, i.e. attenuation rate of 18 dB per octave and cut off frequency of 39 Hz, (3 dB) was used. The amplitude of the integrated EMG signal at the end of the stimulus tone was measured and expressed in percentage of the maximum response amplitude obtained in each session. These relative values were plotted as a function of stimulus intensity to yield stimulus-response curves. Such curves were found to be a reproducible measure of the EMG activity of the stapedius muscle. Fig. 2 shows two stimulus-response curves based on integrated electromyograms obtained in one subject with about 20 min interval during the same

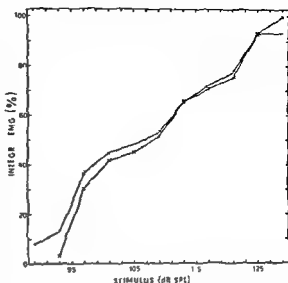


Fig. 2. Two stimulus-response curves for integrated EMG.

experimental session. Each value is the average of two measurements. Contralateral stimulation with pure tone of 0.5 kHz.

experimental session. It is seen that the curves correlate closely.

The average of stimulus-response curves was calculated in the following way. The sound intensity giving 50% of maximum amplitude for each curve was chosen as reference and given the value zero dB. For each 10% of amplitude the corresponding intensity was determined and expressed in relation to the value giving 50%. Thus for each stimulus-response curve 10 dB values were obtained corresponding to each 10% amplitude from 10 to 100%. The average dB-value for each 10% amplitude was then calculated for the group of curves being considered, thus defining the shape of the average curve. The position of the curve was obtained as the average sound pressure level corresponding to the 50% response amplitude for the sample of curves.

In the control group the difference between ipsilateral and contralateral stimulus-response curves was calculated for each subject. The individual differences in intensity giving 10, 20, 30% etc. up to 90% of the maximum response for each curve were determined and the average value was calculated. 10% difference.



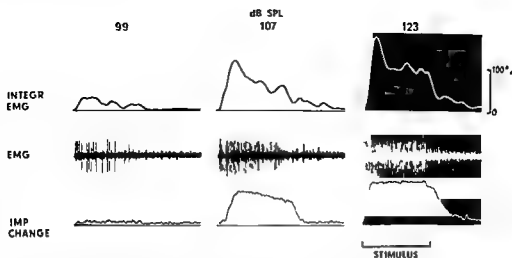


Fig 3 Simultaneous recordings of EMG of the stapedius muscle of the left ear (middle row) and impedance change in the right ear (bottom row) in response to 2 kHz pure tone stimulation of one second duration in right ear. Integrated EMG (rectified and low pass filtered at 39

Hz) is illustrated in the upper row. Vertical scale shows integrated EMG and impedance change amplitude as per cent of maximum obtained value as measured at the end of the stimulus. One subject.

## RESULTS

### A General characteristics

Simultaneous recordings of EMG of the stapedius muscle of one ear and change of the acoustic impedance of the other ear were made. Fig 3 shows the course of the stapedius activity elicited by a 2.0 kHz pure tone at three different intensity levels in one subject. The upper row shows the integrated (rectified and low pass filtered) EMG, the middle row the EMG signal, and the bottom row the change of the acoustic impedance at 800 Hz. At the lowest stimulus intensity (99 dB SPL) the EMG activity seen probably represents only one motor unit. A small impedance change is also seen. The firing frequency of the motor unit is initially high, but declines after about 300 msec. At the higher intensity levels shown in Fig 3 several motor units are activated and the impedance change is more prominent.

Especially at the highest intensity illustrated in Fig 3 the EMG and the integrated EMG as well exhibit an initial peak which soon takes on a lower, fairly stable level. In the impedance change on the other hand there is no or only a very slight tendency to an initial peak. After the end of the stimulus there is an afterdischarge in the

EMG during about 400 msec at the two highest stimulus intensity levels. Fig 3 also shows the slow return of the impedance change to zero after the end of the stimulus corresponding to the afterdischarge of the EMG.

The latency from the start of the stimulus to the first motor unit potential in general decreased when the stimulus level was raised. The lowest value obtained was 12 msec (stimulus with 2.0 kHz pure tone at 124 dB SPL, 2 msec rise time). Impedance change showed a slow onset, making determinations of latency very uncertain. The results thus show that the stapedius reflex has properties that can be expected from a polysynaptic reflex (cf Borg 1973).

### B Threshold sensitivity

The threshold for EMG activity can be defined as the lowest stimulus sound level giving motor unit potentials. It is more difficult, however, to determine the sound level giving the smallest impedance change. The threshold sensitivity for the two measuring techniques has therefore been compared in the present study on the basis of those sound intensity levels needed to elicit responses equal to 10% of the maximum impedance change and integrated EMG respectively (Møller, 1962). In the 4 subjects in which

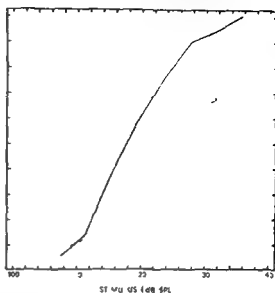
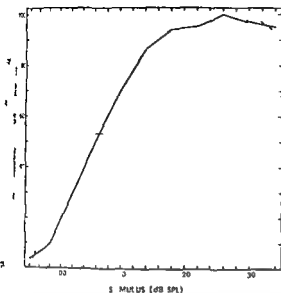


Fig. 4. Stimulus response curves for ipsilateral impedance change (—) and for contralateral integrated EMG (---). Each graph shows results from one subject. The amplitudes of the integrated EMG and of the impedance

change were measured at the end of the stimulus and expressed in per cent of the corresponding maximum obtained amplitude. Stimulus was 0.5 kHz pure tone of 1 sec duration.

Individual comparisons could be made the 10% level of the integrated EMG was reached at a stimulus sound level (0.5 kHz) which was on the average 12 dB below the 10% level of the impedance change. This value represents the difference between the ipsilateral impedance change and the contralateral EMG activity. In the control group of 32 subjects the threshold (10% level) measured as impedance change was 15 dB lower for ipsilateral than for contralateral stimulation with 0.5 kHz pure tone. Ten per cent of the maximum amplitude of the integrated EMG was thus reached at a sound level 3–5 dB below the level giving 10% of maximum impedance change at 800 Hz. Motor unit activity could be recorded on average at another 2 dB lower sound level. The utilized threshold value for impedance change 10% of maximum amplitude was thus reached at a sound intensity 7.8 dB above the level giving the first motor unit activity of the stapedius muscle.

### C. Stimulus-response relations

The amplitudes of the integrated EMG and of the impedance change signal at the end of each stimulus were used as measures of the muscle

activity. Both were expressed in per cent of the corresponding maximum obtained response amplitude. Fig. 4 shows stimulus-response curves for the ipsilateral impedance change (continuous line) and contralateral integrated EMG (broken line) in 2 subjects based on such measurements. It is seen that the amplitude relation between the EMG and the impedance change varied as a function of stimulus intensity. The difference between the curves is in part due to the fact that the impedance change was obtained upon ipsilateral and the EMG upon contralateral sound stimulation. It is also possible that the EMG recorded was not representative of the electrical activity of the whole stapedius muscle but mainly showed the activity of a relatively small region in the vicinity of the recording electrode.

More reliable information on the relation of the total EMG activity of the stapedius muscle and the impedance change ought to be gained from average stimulus response curves based on several subjects. Fig. 5 shows such average curves obtained in the Experimental Group. The broken line illustrates the EMG upon contralateral stimulation. The thin continuous line rep-

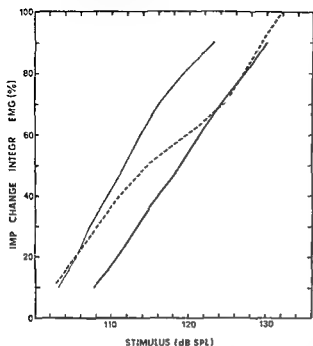


Fig 5 -----, average of the stimulus-response curves for contralateral EMG in the Experimental Group ———, average of stimulus-response curves for ipsilateral impedance change in 4 of the subjects of the Experimental Group ———, average contralateral stimulus-response curves for impedance change as based on the ipsilateral recordings of the 4 subjects of the Experimental Group corrected for the average difference between ipsilateral and contralateral reflex in the control group Stimulus was pure tone at 0.5 kHz of 1 sec duration

resents the impedance change upon ipsilateral stimulation. The heavy continuous line is obtained from the thin line by correcting for the average difference in sensitivity between the ipsilateral and the contralateral reflex in the control group. Thus it represents an estimation of the impedance change of the "EMG ear" upon contralateral sound stimulation and can be directly compared with the broken line. A more stable correlation between these average curves than between the individual curves of Fig 4 is evident. The curves of Fig 5 are approximately rectilinear up to about 130 dB SPL, i.e. more than 20 dB above threshold. The difference between contralateral EMG (broken line), and contralateral impedance change (heavy continuous line) is, at threshold as well as at supra-threshold levels, less than 6 dB.

## DISCUSSION

The results showed that the threshold of stapedius muscle reflex was 5–6 dB higher, measured as change in acoustic impedance at 800 Hz than when measured as integrated EMG. The impedance change and the integrated EMG were both approximately linear functions of stimulus level up to at least 20 dB above threshold. The average difference between two stimulus response-curves was at all times less than 6 dB.

The difference in threshold sensitivity obtained with the two methods was small and regarded to be without clinical importance. Possible explanations for the difference at the lowest degree of EMG activity were accompanied by any pull in the tendon, or the acoustic impedance at 800 Hz was influenced by the weakest change of tension. The latter alternative is supported by the findings by Borg (1968). He showed that the stapedius reflex influences sound at 0.5 kHz at a low level of muscle activity whereas sound at 14 kHz is influenced only well above reflex threshold. Since the measuring tone used (800 Hz) lies between these two frequencies, it might be unaffected by very weak stapedius contraction. Impedance change measurements as a measuring tone frequency would thus perhaps give a more sensitive measure of the stapedius activity at threshold. Peterson & Liden determined the stapedius reflex threshold impedance change at different measuring frequencies, 220, 625 or 800 Hz. The threshold for impedance change at 800 Hz was at a stimulus sound level 2.1 dB above threshold for impedance change at 220 Hz, 3.4 dB below the impedance change at 625 Hz. Since the three values are close to each other, it is likely that all three of the measuring frequencies give threshold values very near that for pull in the stapedius tendon.

It would be interesting if the reflex developed by the stapedius muscle was directly related to the change in impedance.

In rabbits Wersall (1958) found that the relation was a linear function of stimulus sound level within a 30 dB range. At higher levels there was a saturation.

No tension measurements from the stapedius muscle are available in human studies. There are, however, several investigations where the EMG has been correlated to the tension of extremity muscles. Bigland & Lippold (1954) and Stephens & Taylor (1972) for example found a mainly linear relationship between the integrated surface EMG and the tension at isometric contraction. The present results showed that the integrated EMG and the impedance change were both approximately linear functions of stimulus sound level. It thus seems justified to conclude that the impedance change is largely proportional to the tension developed by the stapedius muscle.

#### Clinical aspects

The threshold of the stapedius reflex measured as change in the ear's acoustic impedance is commonly used in clinical diagnosis. It is evidently close to the threshold for contraction of the muscle. In a few clinical studies also the stimulus response curves of the stapedius reflex have been given attention (Klockhoff, 1961; Jørgensen et al., 1967; Peterson & Liden, 1972). Suprathreshold stapedius activity has been used for diagnostic purpose by Burghoff (1968). Anderson et al. (1969), Blom & Zakrisson (1974). Anderson et al. (1969) showed that patients with retrocochlear affections exhibited an abnormally rapid decay of the impedance change during constant low frequency pure tone stimulation.

The present results showed that suprathreshold impedance changes and stimulus-response curves are also good measures of the activity of the stapedius muscle. They can thus probably be used to reveal disturbances in the function of the cochlea, the retrocochlear reflex connections or in the stapedius muscle. This is supported by findings in animal experiments (Borg 1973) where chronic lesions in the brain

response curve of the stapedius reflex. Furthermore, comparisons between ipsilateral and contralateral stimulus-response curves might increase the diagnostic possibilities in disturbances of the lower auditory system.

## ZUSAMMENFASSUNG

Die Impedanzänderung des Ohres bei Beschallung wird in der klinischen Diagnostik weitgehend angewandt und als ihre Ursache wird nunmehr allgemein die Kontraktion des Stapediusmuskels angesehen. Um nähere Daten über die Korrelation zwischen der akustischen Impedanzänderung bei 800 Hz und dem Elektromyogramm (EMG) des Stapediusmuskels zu erhalten, wurden in der vorliegenden Arbeit Untersuchungen an Patienten mit einseitiger Trommelfellperforation durchgeführt. 10% der maximalen Impedanzänderung wurden bei einem Schallpegel von 5 bis 6 dB über dem Pegel erhalten, bei dem die Amplitude des integrierten EMG 10% der Maximalamplitude des integrierten EMG war. Die Impedanzänderung und das integrierte EMG waren beide annäherungsweise lineare Funktionen des Schallpegels bis zu Intensitäten von mindest 20 dB über der Reflektionschwelle. Es ist daraus zu schließen, dass die Bestimmung der Impedanzänderung ein adäquates Mass der Stapediusmuskellaktivität sowohl bei Schwellenpegeln als auch bei überschwelligen Pegeln ergibt. Abschliessend wird auf den Wert derartiger Messungen überschwelliger Impedanzänderungen in der klinischen Diagnostik hingewiesen.

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## FOCUS LOCALIZATION IN TWO CLINICAL TYPES OF OTOSCLEROSIS

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(Received February 15 1974)

**Abstract** In the present study middle ear pathology was investigated in two clinical types of otosclerosis. No difference in localization of otosclerotic foci in the middle ear has been found between these clinical types: tympanical, the other cochlear. Superior and inferior spiral foci are

... cultural involvement is predominantly found in thick complete footplate lesions. A progressive type of otosclerosis can be found in male adolescents. This type shows complete thick footplate invasion without hearing loss. Postoperative hearing gain was satisfactory and to the same degree in both types of clinical otosclerosis regardless the type of middle ear pathology except for round window otosclerosis.

Such has been written about the histopathology of otosclerosis, by far the most of which was in a qualitative descriptive way. Quantitative statements on the frequency and distribution of otosclerotic foci in the bony capsule of the labyrinth have been published by Guild (1944), Nylen (1949) and by Ruedi & Spöndlin (1957).

A correlation between the type of footplate pathology and the type of hearing loss in otosclerosis has initially been assumed by Farnior (1958) but in his later work (Farnior, 1963) he has found no significant correlation between the degree of deafness and the severity of the footplate pathology. This is in agreement with others (Antoli-Candela 1960, House, 1959). Ludman (1962) came to another conclusion. It has been assumed by him that the thickness of the footplate lesion is related to the degree of deafness.

Two clinical types of otosclerosis have been reported by us in a previous study (Marres et al., 1973). One clinical type, which is called type I

otosclerosis, concerns the patients having a relatively moderate hearing loss for all sound frequencies. The other clinical type, designated type II otosclerosis, comprises the patients having a more severe hearing loss and the poorest hearing at the highest frequencies. It is assumed by us that type I otosclerosis is identical with the tympanical type of Politzer-Siebenmann and that type II otosclerosis is identical with the Lermoyez type of cochlear otosclerotic involvement.

In the present report a comparison between the type of pathology in both clinical types of otosclerosis has been made in order to see whether pathology is correlated with type of hearing loss.

## MATERIAL AND METHOD

Middle ear pathology was recorded following the coding scheme by Farnior (1958) in a group of 271 patients with otosclerosis. Hearing loss in both air and bone conduction at the frequencies 0.5, 1, 2, 4 and 8 kHz was measured shortly before stapedectomy and insertion of a Teflon piston prosthesis and also one year after the operation. The coding system is given in Table I.

All calculations were performed with the IBM System/370 model 155 computer of the "Universitair Reken Centrum" at Nijmegen. The frequency of each pathology code was scored in both groups of patients. The combined frequencies of pathology codes were a and tested in a contingency table.

Table 1 The frequencies of the various aspects of otosclerosis, coded according to Farnior (1958)

Modifications codes VIII and 2 denote Farnior's codes VII and VIII respectively

Codes	Type I	Type II	Total
<i>Anatomical situation</i>			
Depth of the footplate			
A1 superficial	78	43	121
2 moderately superficial	76	30	106
3 moderately deep	21	12	33
Angulation of crura			
B1 upright	135	70	205
2 downward tilt (free)	15	14	49
3 downward tilt (fixed)	4	1	5
<i>Pathology</i>			
Footplate otosclerosis			
III1 none	1	0	1
2 anterior	34	12	46
3 posterior	23	9	32
4 superior marginal	42	18	60
5 inferior marginal	55	22	77
6 $\frac{1}{4}$ footplate	15	7	22
7 $\frac{1}{2}$ footplate	18	9	27
8 $\frac{3}{4}$ footplate	13	5	18
9 complete footplate (thin)	42	24	66
10 complete footplate (thick)	32	21	53
Crural otosclerosis			
III1 none	119	55	174
2 anterior crus	24	10	34
3 posterior crus	14	7	21
4 bicrural	22	14	36
Round window otosclerosis			
IV1 none	107	46	153
2 marginal without closure	1	1	2
3 partial closure	0	2	2
Thickness of otosclerosis			
V1 very thin	91	42	133
2 thin	51	20	71
3 moderately thick	38	23	61
Colour of otosclerosis			
VII1 white	116	60	176
2 nearly white	48	19	67
3 pink	11	5	16
Miscellaneous pathology			
VIII1 mucous membrane webs	6	5	11
2 fibrous adhesions	5	0	5

test) against the expected frequencies that could be calculated. Trivial combinations which were the direct consequence of a certain excess in the coding system, were ignored. A "significant combination" is defined as a combination,

which incidence has been proved to be significantly higher than could be expected and is not ascribed to chance alone other than 5% of the cases.

Special interrelations between pathology coding items have been sought. The relations of age and sex have been considered in connection with the type of hearing loss, the type of middle ear pathology and the hearing impairment experienced by the patient. Miscellaneous pathology is presented and special attention is paid to the patients with a very severe hearing loss.

## RESULT

### Frequencies of otosclerotic foci

The frequency of each pathology code in the operation files is given in Table 1 for both type I and II patients. There is no significant difference between the frequencies of the codes in type I and II patients, nor in males and females (not shown here). In view of this, most of the following is about all categories of pathology in type I and II, males and females together.

The incidence of total footplate otosclerosis (III9 and III10 in Farnior's coding system, Table 1) is high, which indicates that we are dealing with extensive lesions. Round window otosclerosis was very seldom seen (2.5% of the cases).

### Combined frequencies of foci

A relevant part of the computer generated table is shown in Table II. From the observations presented in this table, which proved to be significant, the following conclusions can be drawn.

1 In the deeper placed stapes footplates (A2, 3) more downward tilted crura (B2, 3) are found and more superior (II4) or inferior (II5) marginal footplate otosclerosis.

2 Superior (II4) and inferior (II5) marginal footplate otosclerosis are significantly associated. They are found in combination in about half of the cases.

3 Few crural involvement, i.e. III1, is found in combination with marginal footplate otosclerosis (II4, 5) or in very thin (VI1) otosclerosis.

Table II Combined frequencies

	A2,3	B2,3	II2	3	4	5	10	III1	2	3	4	VI	VII
23	139												
23	43	54											
2			46										
3				32									
4	43				60								
5	54				42	77							
0							53						
1					50	63		174					
2			17						34				
3				12						21			
4							20				36		
1								107				133	
1							44						176

i Anterior crus otosclerosis (III2) is associated with anterior pole otosclerosis (II2) as is anterior crus otosclerosis (III3) with posterior footplate otosclerosis (II3)

i Bicurral involvement (III4) is predominant—found in combination with thick complete footplate lesions (II10)

i Complete thick footplate otosclerosis (II10) in most instances has a white aspect (VI1)

#### Footplate pathology

From the previous findings it can be deduced that the most common types of footplate pathology are

1. Polar otosclerosis (26% of the patients) at anterior (II2) or the posterior (II3) pole of footplate, sometimes at both poles (10% of cases) and in 1/3 of the cases involvement of the associated crus (III2 or III3 respectively)

2. Marginal otosclerosis (35% of the patients) at the superior (II4) or at the inferior (II5) margin and in half of the cases at both margins of footplate. In marginal otosclerosis in most instances (about 85% of the cases) both crura are intact (III1)

3. Complete footplate otosclerosis (44% of patients) in 55% of the cases thin (II9), in other cases thick (II10) and in those cases preferentially white (VI1, in 83% of the cases)

and in about 1/3 of the cases with bicurral involvement (III4)

Overlapping of type 1 and 2 pathology, mixed polar and marginal otosclerosis, was present in 7% of the patients, extending to fully circumferential otosclerosis (II2, 3, 4, 5) in 1%.

In Table III the incidence of the various types of footplate pathology is shown for the different clinical types of otosclerosis. From this table it can be seen that no significant difference exists between the type of footplate pathology in the two clinical types.

#### Footplate pathology regarding clinical type, sex and age

The only significant difference in footplate pathology between the sexes lies in the combined frequency of superior marginal (II4) and inferior marginal (II5) otosclerosis. This combination occurs almost twice as much in females (see Table IV). In fact the high incidence of this combination in females is responsible for the significance of this combination as mentioned before.

Other types of footplate pathology are equally distributed with respect to the sexes and age classes, with only one important exception. In a group of 12 boys, 14–19 years old, 7 had thick complete footplate otosclerosis (II10) and



Table III The incidence of the various types of footplate pathology in the two clinical types of otosclerosis

Footplate otosclerosis	Type I	Type II	Total
None	1	0	1
1 Polar	52	18	70
2 Marginal	65	30	95
3 Complete	74	45	119
1,2 mixed	13	6	19
Unspecified	4	1	5
No. of patients	183	88	271

had type I hearing loss (the boy with type II hearing loss having round window otosclerosis with partial closure, i.e. IV3). The high incidence of type I hearing loss and thick complete footplate otosclerosis are very significant in this group of young males, as is the combined incidence of these two characteristics. The young male group itself is a significant finding in the whole age distribution of the patients. It might be a very progressive type of otosclerosis with a high incidence of thick footplate lesions, but without perceptive loss (i.e. type I hearing loss).

*Result as related with clinical type, of pathology, sex and age*

As shown in Table V, there is no relation between prognosis and clinical type of otosclerosis, sex, type of footplate pathology or age. Hearing gain is good in all categories and the average gain in the Fletcher-index one year after the operation is 32 dB. In miscellaneous pathology hearing gain is identical. This is not the case in round window otosclerosis, however. Only one of the (four) patients with round window

Table IV The combined frequencies of superior marginal (II4) and inferior marginal (II5) footplate otosclerosis in males and females

Combinations	Males	Females	Total
Realized	12	30	42
Not realized	13	5	18
Maximum number, which could have been realized	25	35	60

Table V. The mean hearing gain at the frequencies in type I and II patients with females of various age classes, in relation of footplate pathology

Age class (yrs)	Type I Footplate lesions						Type II					
	Complete						Complete					
	Thin		Thick		Minor		Thin		Thick		V...	
	m	f	m	f	m	f	m	f	m	f	m	f
0-24	33	27	29	31	33	29	—	—	—	—	—	—
25-39	36	28	31	27	34	32	38	58	(24)(18)	31	—	—
40-75	31	32	—	29	26	32	39	38	35	32	30	—

Average gain 32 dB

otosclerosis (having IV2, marginal without c) had sufficient hearing gain. This patient was only one having round window otosclerosis combined with type I hearing loss. The patients with round window otosclerosis (IV2 and two IV3, partial closure) had hearing loss and even for that type of they had extremely severe hearing loss. Patients had hearing loss (1) or poor gain (2) one year after the operation. For these patients the shift in the Fletcher-index after the operation was significantly smaller compared with the more regular type II. Although the incidence of round window otosclerosis was too low for statistical testing, it seems to be associated with severe perceptive involvement. It seems to have a poor prognosis, because it can be associated with extensive sclerosis with cochlear involvement (Fletcher 1960).

*Footplate pathology and very severe hearing loss*  
In the group of patients with a very severe hearing loss (21 after elimination of 3 patients

# Table VI Survey of the literature on macroscopic footplate lesions in bony stapes craniolysis compared with present study

Incidence of percentage of ears with stapes ankylosis	small lesions			moderate lesions					extensive lesions		
	1	2	3	4	5	6	7	8	9	10	11
	several hundred			1937	125	78	7000	28	35	102	271
Footplate pathology (%)											
Crural lesion	36	—	—	8	—	9	—	—	—	33	0.3
Anterior footplate	41	75	68		70	63	58	24	29	—	19
Posterior footplate	14	20	14			4	20	75	8	16	28
Anterior polar	—	—	18	16	40	19	22	1	83	51	44
Marginal footplate	9	7	—								

References: 1 Bellucci, 1958, 2 Farnior, 1960, 3 Fleischer, 1958, 4 Antoli-Candela, 1960, 5 Farnior, 1963, 6 García-Ibáñez & Jurato, 1969, 7 Kaplan & Shambaugh, 1961, 8 Rüedi & Spoendlin, 1957, 9 del Bo & Bergomi, 1970, 10 Davies & Jackson, 1962, 11 present study.

and window otosclerosis) half of the patients (33) above 50 years of age are present. There is no difference in footplate pathology between the 6 patients and the other 7 in the same age group, which had relatively moderate hearing loss before the operation. A normal hearing gain was experienced by these 6 patients as well as all others in the group with very severe hearing loss.

## DISCUSSION

### Types of footplate pathology

Types of footplate pathology as reported in the literature, which could be reclassified on the basis of the present classification (see Table III) are listed in Table VI. Only the selected cases reported by del Bo & Bergomi (1970) and those of Davies & Jackson (1962) show a higher incidence of extensive lesions than reported in the present paper (see Table VI).

### Combination and development of foci

Isolation of the nearby crus from a polar focus, most often anteropolar, is a common finding (Antoli-Candela, 1960; Bellucci, 1958; Farnior, 1960, 1963; García-Ibáñez & Jurato, 1969). In the present paper, though more posterior involvement is reported here. The predominance of bicrural involvement in the more affected

footplate as presented here, has also been reported by Antoli-Candela (1960), Bellucci (1958) and by Farnior (1960). The tendency toward bicrural involvement in inferior marginal footplate otosclerosis as reported by Farnior (1960) could not be shown in the present study. On the contrary, it is a significant finding that few crural involvement was seen in marginal otosclerosis. It cannot be confirmed that a deeply placed footplate with downward tilted crura predisposes toward crural or complete otosclerosis (Farnior, 1958, 1960). Indeed, more downward tilted crura and more marginal (but not more extensive than that) otosclerosis were found in the deeper placed footplate but, as stated before, in fact few crural involvement in combination with this marginal otosclerosis was seen.

The findings in early lesions as reported in the literature and the present findings suggest the following general "scheme of development" of the footplate lesions.

A primary site of affection seems to be the anterior footplate pole, though other sites might be the posterior pole or one or both margins as well. Extension of the primary lesions can take place in all directions, in many instances leading to crural involvement as soon as the nearby pole is involved.

Types of pathology and types of hearing loss

The fact that no difference in middle ear pathology has been found between type I and type II otosclerosis implies that the difference between these types of otosclerosis must be sought outside the middle ear. It can be shown that besides middle ear pathology type I and II patients have one parameter in common: the airborne gap. In both groups of patients this parameter has the same value before the operation. Moreover, the reduction in air bone gap or hearing gain after the operation is exactly the same in both groups. Even in the type II patients with a very severe hearing loss before the operation, round window otosclerosis excluded. Apart from the stapes fixation with the concomitant conduction loss, type II otosclerosis probably has cochlear involvement leading to additional perceptive hearing loss.

ZUSAMMENFASSUNG

Bei Patienten mit zwei klinisch verschiedenen Arten der Otosklerose, bei denen Stapedektomien vorgenommen waren, wurde die Pathologie des Mittelohres untersucht. Die Lokalisation der otosklerotischen Herde im Mittelohr zeigte bei beiden Typen — dem tympanischen oder cochleären — keinen Unterschied. Bei der Fussplattenototisklerose wurde besonders bei Frauen öfters eine Kombination von Herden am oberen und unteren Rand gefunden. Bei dieser Lokalisation wurden wenig kraniale Formen beobachtet. Die Crura waren meistens durch Ausbreitung des Prozesses vom nachstgelegenen Pol aus betroffen. Beteiligung beider Crura am Krankheitsprozess wurde hauptsächlich bei totaler Fussplattenototisklerose gefunden. Bei männlichen Adoleszenten zeigte sich ein progressiver Typ von Otosklerose, wobei eine dicke, total befallene Fussplatte ohne Perzeptionsverlust vorlag. Bei beiden klinischen und bei allen beschriebenen pathologischen Typen von Otosklerose war der postoperative Hörgewinn gleich gut. Ausgenommen waren lediglich die Patienten mit Herden am runden Fenster.

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# SCHÄDIGUNG DES INNENOHRES NACH TYMPANOPLASTIK

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(Eingegangen am 25. Januar 1974)

**Zusammenfassung:** Die Auswertung der Tonaudiogramme von 930 Tympanoplastiken Typ I, die in den Jahren 1960-1972 an der Freiburger Univ. Hals-Nasen-Ohrenklinik operiert wurden, zeigt 3 Wochen nach der Operation eine Innenohrschädigung bei 17%, 3 Monate nach dem Eingriff noch eine solche bei 10,8% der Patienten. Als Ursache dieser Innenohrstorung werden diskutiert:

1. Die Traumatisierung des Innenohres während der Operation
2. Die Tatsache, dass die Operation oft am kranken bzw. krankgewesenen Ohr erfolgt
3. Die individuelle Empfindlichkeit des Ohres gegen Belastung.

Bald nach Entwicklung der Tympanoplastik wird über Einzelfälle einer Schädigung des Innenohres, dokumentiert durch ein postoperatives Absinken der Knochenleitungsschwellenkurve evtl. bis zur Ertaubung berichtet (Beickert, 1958, 1962, Schweiz, 1957, 1958). Mehrere Veröffentlichungen bestätigen diese Erfahrung (Bandelow, 1960, Bartual, 1964, Heerwagen, 1960, Palva et al., 1973, Pfaltz et al., 1962, Rector, 1964, Rentzsch, 1973, Wullstein, 1968), wobei besonders die Tympanoplastiken der Typen III und IV betroffen sind. Daneben wird betont, dass auch bei Typ I eine Beeinträchtigung der Innenohrfunktion möglich ist (Ballantyne, 1970, Beickert, 1958, 1962, Bicknell, 1971, Coley, 1969, Thornburn, 1971). Eine Reaktion des Innenohres bei diesem einfachsten Typ der Tympanoplastik ist erstaunlich und nur schwer zu erklären. Wird doch hier im Gegensatz zu den ausgedehnten Eingriffen der Typen II, III und IV mit Revision der Fenster, Einlage von Metall oder Plastikfolie Stellen einer Colu-

mella u. a. ruft eine Trommelfellperforation hervor.

Ausgehend von diesen Überlegungen stellen wir uns vor allem drei Fragen, deren Beantwortung wir nach Auswertung unseres eigenen Materials erhofften:

1. Tritt nach Tympanoplastik Typ I eine Innenohrstorung auf?
2. Wie häufig ist sie?
3. Was sind die möglichen Ursachen?

Die Eingriffe wurden von verschiedenen Operateuren nach der prinzipiell gleichen Technik durchgeführt. Nach Darstellung der Trommelfellperforation, evtl. durch Gehörgangserweiterung und nach Kontrolle der Beweglichkeit der Gehörknöchelchenkette, wurde die Perforation mit Temporalfascie oder Fascia lata unterfüttert. Überprüft wurden die Unterlagen von 930 an der Freiburger Hals-Nasen-Ohrenklinik in den Jahren 1960 bis 1972 operierten Tympanoplastiken vom Typ I, von denen wir 3 Tonaudiogramme vor der Operation, 3 Wochen und 3 Monate postop. auswerten. Für die Durchschnittsberechnung des Hörverlustes in dB dienen die Knochenleitungswerte bei den Frequenzen 500, 1000, 2000 und 4000 Hz, wobei aus der erhaltenen Summe das arithmetische Mittel gebildet wurde.

## ERGEBNISSE

**Verhalten der Knochenleitungsschwellenkurve nach Tympanoplastik Typ I**

- a) 3 Wochen nach Operation (Tabelle 1). 930 Tympanoplastiken Typ I war bei

Tabelle I Verhalten der Knochenleitungsschwellenkurve bei 930 Tympanoplastiken Typ I 3 Wochen postop im Vergleich zum praeeoperativen Verlauf

Zahl der Op	KL Schwellenkurve	°
757	Unverändert	81,4
158	Verschlechtert	17,0
15	Gebessert	1,6

Knochenleitungsschwellenkurve gegenüber dem praeeoperativen Stand unverändert 158 = 17%, zeigten eine deutliche Verschlechterung dieser Schwellenkurve und bei 15 war eine Besserung gegenüber vor der Operation zu beobachten. Der durchschnittliche Knochenleitungsabfall betrug zu diesem Zeitpunkt 10,34 dB, die postoperative Besserung der Innenohrleistung durchschnittlich 12,23 dB gegenüber dem praeeoperativen Stand (bei 15 Fällen = 1,6%).

b) 3 Monate nach Operation 19 Patienten waren zur Nachuntersuchung nicht mehr erschienen, so dass hier nur noch 139 ausgewertet werden konnten (Tabelle II). Davon hatte bei Patienten die Knochenleitungsschwellenkurve

Ausgangswert wieder erreicht. Bei 66 Patienten war eine Besserung gegenüber dem Zustand von 3 Wochen postop festzustellen, bei 8 war keine Änderung eingetreten und bei 27 liess sich eine weitere Verschlechterung aufzeigen. Der durchschnittliche Knochenleitungsverlust betrug 4,86 dB.

Die Nachkontrolle zeigt, dass sich in 27,4%

Tabelle II Verhalten der Knochenleitungsschwellenkurve 3 Monate postop bei 139 Tympanoplastiken Typ I, bei denen 3 Wochen nach dem Eingriff eine Verschlechterung der Knochenleitung festgestellt wurde

Zahl der Op	KL Schwellenkurve	°
38	Wieder erreicht	27,4
66	Gebessert	47,6
8	Unverändert	4,8
27	Weiter verschlechtert	19,2

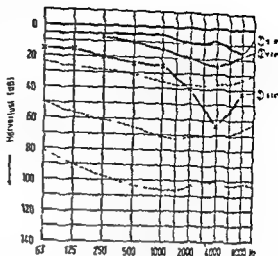


Abb 1 Knochenleitungsschwellenkurve nach Tympanoplastik Typ I. Deutliches Absinken der Schwelle nach der Operation. Nur teilweise Erholung bei der Nachkontrolle.

der Fälle der ursprünglich vorhandene postoperative Knochenleitungsverlust wieder normalisiert hat. Bezogen auf die Gesamtzahl von 930 operierten Ohren bleibt 3 Monate nach der Operation eine Innenohrschädigung von 10,8%.

Zu ergänzen ist, dass sich unter den 930 Plastiken Typ I eine Ertaubung fand, die 2 Jahre nach der Operation auftrat und so wahrscheinlich nicht in unmittelbarem Zusammenhang mit der Operation steht.

Der postoperative Knochenleitungsverlust machte sich in allen Frequenzen bemerkbar, doch waren grundsätzlich die höheren Frequenzen von 2000 bis 8000 Hz stärker von der Innenohrschädigung betroffen (Abb 1).

Geschlechtsspezifische Innenohrreaktionen konnten wir ebenso wenig feststellen wie vom Alter abhängige — unsere Patienten waren zwischen 7 und 70 Jahre alt — Schädigungen des Innenohres. Bei Überprüfung der Unterlebens zeigte sich zwar, dass direkt nach der Operation der Knochenleitungsverlust bei älteren Patienten gegenüber den jugendlichen etwas deutlicher war, dass sich aber 3 Monate nach der Operation kein Unterschied mehr fand.

#### Patienten mit mehrmaliger Tympanoplastik Typ I am gleichen Ohr

Bei 28 Patienten wurde die Plastik Typ I aufgrund einer Sekundärperforation einmal wieder

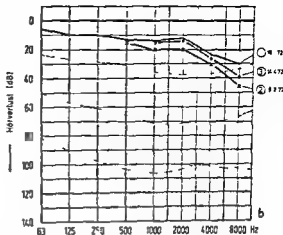
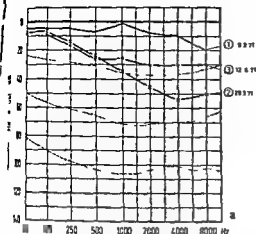


Abb. 2. Pat. H P a) Tympanoplastik Typ I h. Ohr (Frühjahr 1971) Deutliches Absinken der Knochenleitungsschwellenkurve postop und partielle Erholung 3 Monate später b) Tympanoplastik Typ I re. Ohr (Frühjahr 1972)

Geringes Absinken der Knochenleitungsschwellenkurve nach dem Eingriff. Bei der Nachkontrolle Ausgangswert wieder erreicht.

bolt. Nach der Operation besserte sich die Knochenleitung in 5 Fällen = 17,8% um durchschnittlich 4,2 dB, unverändert waren 8 = 28,6% und verschlechtert hatte sich die Hörleistung über Knochenleitung bei 5 Patienten = 23,6%. Im durchschnittlich 9,5 dB. Bei 3 Patienten wurde die Tympanoplastik Typ I dreimal durchgeführt. Von diesen Patienten war die Knochenleitung bis zu 1 Jahr nach der dritten Operation bei einem unverändert geblieben, bei den beiden anderen hatte sie sich um durchschnittlich 3 dB verschlechtert. Die Ergebnisse zeigen, dass Mehrfachoperationen auch bei Tympanoplastik Typ I ein Risiko für das Innenohr darstellen.

nissen nach einem Innenohrabbau auf dem einen Ohr die nachfolgende Operation des Gegenohres nicht zwangsläufig zu einem Knochenleitungsverlust führt.

#### Vorgeschädigte Ohren

Auch die Frage, ob vorgeschädigte Ohren im Hinblick auf die postoperative Erholung der Knochenleitung ein anderes Verhalten als präoperativ normale Innenohren zeigen, können wir verneinen. Zu den vorgeschädigten Innenohren zählten wir jene, bei denen präoperativ eine deutlich ausgeprägte c<sup>3</sup>-Senke im Tonaudiogramm vorlag.

#### Beidseits operierte Ohren

Bei 40 Patienten wurde auf beiden Ohren eine Tympanoplastik Typ I durchgeführt. 5 Patienten zeigten nach der Operation auf dem einen Ohr einen Knochenleitungsverlust. Das Gegenohr blieb nach der Trommelfellplastik ohne Innenohrschädigung (Abb. 2). Bei einer anderen Gruppe von 6 Patienten war zwar nach der ersten Operation kein Knochenleitungsverlust festzustellen, beim Gegenohr trat dieser jedoch nach erfolgter Operation auf. Nur in 2 Fällen konnte nach beidseitiger Operation auf jedem Ohr eine Verschlechterung der Innenohrleistung registriert werden, so dass nach unseren Ergeb-

zwischen 500 und 4000 Hz bei diesen Ohren ein um etwa 4 dB starkerer Knochenleitungsverlust als bei den nicht vorgeschädigten Innenohren vorhanden war. Bei der Kontrolle 3 Monate nach Operation war dann kein Unterschied mehr zwischen vorgeschädigten und präoperativ normalen Innenohren festzustellen. Wir sind daher der Auffassung, dass eine Vorschädigung ohne wesentliche Bedeutung ist, da auch hier nach entsprechender Wartezeit gleiches Knochenleitungsverhalten zwischen vorgeschädigten und nicht vorgeschädigten Innenohren vorliegt.

# DISKUSSION

Aufgrund unserer Untersuchungen müssen wir feststellen, dass nach Tympanoplastik Typ I eine Innenohrschädigung unterschiedlichen Grades auftreten kann 3 Wochen postop war sie bei 17% unserer Patienten festzustellen, 3 Monate nach dem Eingriff noch bei 10,8%. Wir können so die Ergebnisse von Bicknell (1971), 14%, sowie Thornburn (1971), 9%, bestätigen Befallen waren alle Frequenzen, besonders bevorzugt die höheren ab 2000 Hz Hervorzuheben ist, dass bei 1/4 der Patienten, die 3 Monate nach dem Eingriff noch eine Innenohrschädigung aufwiesen, die Hörverschlechterung 30 dB und mehr betrug

Zu beantworten bleibt die Frage nach den Ursachen dieser Beeinträchtigung der Innenohrfunktion nach Tympanoplastik Typ I Entscheidend für die Entstehung einer Innenohrschädigung bei diesem Typ der Tympanoplastik sind u E vor allem folgende Faktoren

1 Die Tatsache, dass wir oft an einem kranken oder krank gewesenem Ohr operieren, wobei auch Heermann (1960) bereits hingewiesen

Ein Wiederauftreten einer entzündlichen Reaktion lässt sich nicht voraussehen und auch nicht vermeiden Sie mag möglicherweise durch den Eingriff selbst ausgelöst sein

2 Für besonders wichtig halten wir die operative Belastung, worauf auch Kley (1969) und Schweiz (1957, 1958) sowie in jungster Zeit Palva hinweisen Manipulationen an der Gehörknöchelchenkette wie Kurzung des Hammergriffs, um iatrogene Cholesteatome zu vermeiden, oder die Prüfung der Beweglichkeit der Gehörknöchelchenkette führen zu unphysiologischen Schwingungen des Steigbügels, worauf Tonndorf (1973) erst vor kurzem hingewiesen hat Wir müssen dabei mit einer mechanischen Irritation des Innenohres rechnen, die mit dem akustischen Trauma vergleichbar ist (Palva et al, 1973)

Folgen dieser mechanischen Irritation des Innenohres sind metabolische Störungen So konnte Schmieder (1972) nach experimentellen Stapediolyse eine Erweisentladung beobachten, und Schatzle & Haubrich (1971) stellten im

Tierexperiment nach Stapedektomie Ferrer Schädigungen im Bereich der Sinneszellen & Basalwindung der Cochlea fest Diese Störschädigungen werden im Tierexperiment 3 bis 4 postoperativem Tag merkbar und bis sich in vielen Fällen bis zum 28. Tag nach dem Eingriff wieder zurück, ähnlich wie wir es früer nach akustischem Trauma aufzeigen konnten (Beck & Michler, 1960) Dies erklärt das Wiederanstiegen der Knochenleitungsschwelle in einem späteren postoperativen Zeitraum Das weitere Absinken der Knochenleitung in einzelnen Fällen kann darauf zurückgeführt werden dass u ein Teil der geschädigten Haarzellen im Cortischen Organ nicht wieder erholt, sondern grundgelegt Es handelt sich hier um biologische Reaktionen, mit denen auch beim Typ I d Tympanoplastik zu rechnen ist Für die wahrscheinliche Rolle einer mechanischen Irritation und der durch ausgelösten metabolischen Störung in der Cochlea nach Tympanoplastik Typ I sprechen auch unsere Beobachtung, dass nach Mehrfachoperationen und damit nach mehrmaliger Traumatisierung des Innenohres ein deutlicher Knochenleitungsabfall zu beobachten war

3 Von Wichtigkeit ist weiter die individuelle Empfindlichkeit des Innenohres auf fördernde Operationen (Wullstein, 1968, Batsch, 1964, Schmieder, 1972, Schweiz 1958) Für die Auslösung des Schadens kann jedoch nach unseren Untersuchungen keine grundsätzliche Bedeutung zukommen Müssen doch nach Schmieder (1972), beide Ohr gleichermassen auf den operativen Eingriff reagieren So wäre bei beidseits operierten Patienten auch ein beidseitiger Innenohrschaden zu erwarten Bei unseren beidseits operierten Patienten konnten wir dagegen feststellen, dass ein Knochenleitungsabfall bei Operationen des ersten Ohres bei Operation des Gegenohres nicht auftreten muss Von 40 Fällen war dies nur bei 1 Patienten der Fall

Beim Ausmass der geschilderten Folgen der mechanischen Irritation ist allerdings der individuelle Empfindlichkeit u E eine wesentliche Rolle zuzuweisen Jeder Operateur weiss, dass nach intraoperativer Traumatisierung kein

Innenohrreaktion eintreten kann, während bei Eingriffen, bei denen man glaubt, besonders schädigend vorgegangen zu sein, eine postoperative Innenohrschädigung zu beobachten ist.

Eine Vorschädigung des Innenohres (Boenninghaus, 1962, Schwetz, 1957, 1958) dürfte hierin nach unserer Untersuchung ebenso wie Alter und Geschlecht keine wesentliche Rolle spielen.

## SUMMARY

We have evaluated the audiograms of tympanoplasties which were operated upon between 1960 and 1972 in the ENT Clinic in Freiburg. In 17% of cases, the inner ear turned out to be impaired, 3 weeks after the operation. Three months after the tympanoplasty 10.8% of the inner ear proved to be damaged. We have discussed the various causes of these impairments: 1. During the operation the inner ear can become traumatised. 2. Operations are often performed on impaired ears. 3. There is an individual degree of vulnerability in each ear.

## LITERATUR

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## MIDDLE EAR MECHANICS AND ALTERNOBARIC VERTIGO

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(Received January 23, 1974)

**Abstract** The effects of overpressure induced locally in the middle ear through free air communication between the middle ear and the external ear canal were compared with the effects of a relative overpressure of the same magnitude induced indirectly by lowering the ambient pressure. The same vestibular response was seen and the main condition for inner ear stimulation was the fact that the overpressure should reach a certain level. However, a certain pressure difference between the ears is also involved. The overpressure seemed to affect the inner ear without involvement of the eardrum, and merely a movement of the eardrum could not be shown to affect the inner ear. The possibility of caloric stimulation due to temperature changes of the middle ear gas volume was also considered and excluded.

**Introduction** Vertigo (A V), i.e. vertigo due to increased pressure in the middle ear, has been studied by the present author in cooperation with Ingelstedt & Ivarsson (1974) in an experimental study on man. It was found possible to elicit A V in certain subjects when exposed to simulated ascents during which the subjects were told to avoid active equilibration. There was then no clearing of the ears until the relative overpressure in the middle ears was high enough to force the Eustachian tubes open passively.

The way in which this relative overpressure affects the inner ear is, however, still unknown. The mechanism has been assumed to be due to anatomical abnormalities such as a thin bony wall between the middle ear and the inner ear (Melvill Jones, 1957, Benson 1965) or an un-

usual oval and round window anatomy" (Bos et al., 1964). Vertigo and nystagmus caused by overpressure in the middle ear were however also demonstrated by Nylén & Karlfors (1921). They induced an overpressure directly in the middle ear in subjects with an eardrum perforation due to a chronic or acute otitis media. They found that a pressure of at least 40-60 mmHg was required for the vestibular response to appear, as a rule, however, 100 up to 150 mmHg or more. Yet Ingelstedt et al. (1974) could show that it is possible to elicit vertigo in otologically healthy subjects also. They could elicit vertigo in 5 out of 79 subjects but these subjects had a forcing pressure on one side of a much higher level than the mean forcing pressure of the material.

Vestibular responses to ambient pressure changes have also been supposed to be due to sudden movements of the eardrum system (eardrum and the ossicular chain) causing a fluid displacement in the inner ear (Melvill Jones, 1957, Fields, 1958, Gregg & Ferrel 1967). These suggestions, however, are not based on experiments. Such sudden movements are thought to occur at the moment of pressure regulation of over- and underpressure in the middle ear or at the moment of a positive Valsalva's manoeuvre. The footplate of the stapes may be expected to move in the same direction as the eardrum and it is suggested that large displacements of the eardrum might cause fluid displacement in the inner ear through the leakage between the stapes and the eardrum system.

This investigation was supported by grants from the Swedish Medical Research Council (No. B 73 17X 99-09) and the Swedish Delegation for Applied Defence Research (Project Nos. U 87/1972 and U 95 1973).

experiments, however, have been performed on simulated ascents and descents of 90 cm (corresponding to about 800 m ground level) at a speed of 4 cmH<sub>2</sub>O/sec (about 1 m/sec), during which the subjects actively varied the middle ear pressure, thus causing large displacements of the eardrum. No ear stimulation could be recorded during the experiments (Ingelstedt et al., 1974). Yet other experiments on the same subjects these authors were able to show that it was possible to elicit AV without sudden pressure changes. They found a vestibular stimulation starting with a relative slow pressure increase in the middle ear (4 cmH<sub>2</sub>O/sec), but the stimulation could not be recorded until the relative overpressure reached a certain level (about 60 cmH<sub>2</sub>O). Asymmetry between the ears owing to different forcing pressures could also be observed.

The aim of the present study was to investigate

(a) if the vestibular stimulation is elicited by movements of the eardrum when the relative overpressure in the middle ear displaces the eardrum outwards, thereby moving the stapes anteriorly

(b) if the relative overpressure in the middle ear acts upon the inner ear directly without involvement of eardrum movements

## MATERIAL

The material consisted of 2 subjects, 23 and 25 years old. The subjects were regarded as otologically normal with no history of ear disease and a normal ear examination. The hearing threshold was within 0–20 dB (related to ISO standard 1964). The subjects were free from signs of catarrhal infection and there was no story of labyrinthine disease. The vestibular function was tested by caloric irrigation and ENG, and was found normal. Only 2 individuals were examined since it was difficult to find suitable subjects. The criteria for the selection of the subjects were as follows:

Otologically healthy, high forcing pressure on one side, appearance of vertigo when exposed to simulated ascents and passive clearing of the ears

Subjects with the above mentioned qualities had to agree to myringotomy and application of a transmyringal plastic tube

The subjects were selected from the material of another study, presented by Ingelstedt et al. (1974), (subjects I and II)

## EQUIPMENT AND METHOD

The following symbols are used

$P_{atm}$	atmospheric pressure on ground
$P_m$	pressure in middle ear
$P_{tm}$	pressure gradient across eardrum
$P_{ch}$	pressure in chamber, i.e. ambient pressure
$P_{ec}$	pressure in external ear canal
$V_m$	volume of airfilled middle ear space
$V_{tm}$	volume displacement of eardrum in relation to its neutral position
$V_{muc}$	volume of mucous membrane lining the middle ear space
$V_{tm}$	airflow through resistor of the ear canal flow meter, caused by volume displacement of eardrum
$V_{ac}$	airflow through resistor of the ear canal flow meter, caused by expansion and compression of gas volume in external ear canal and in flowmeter system by changing the ambient pressure
$V_{ref}$	airflow through resistor of the reference flowmeter, caused by expansion and compression of gas volume in reference system by changing the ambient pressure

$\Delta$  before the symbol indicates a change of the variable. Pressure is expressed in cmH<sub>2</sub>O, volume in microlitre ( $\mu$ l) or millilitre (ml), and airflow in microlitre/sec ( $\mu$ l/sec).

$P_m$ ,  $P_{ch}$  and  $P_{ec}$  are relative to atmospheric pressure on the ground

$P_{md}$  middle ear pressure minus the pressure of saturated water vapour at 37°C, this being regarded as non-compressible according to Boyle's law

The effect of overpressure in the middle ear was studied by increasing the pressure in the middle ear in different ways, indirectly and directly

### 1 Pressure gradient across an intact eardrum by changing ambient pressure

Fig 1 gives an outline of the equipment used for the recordings. The middle ear pressure

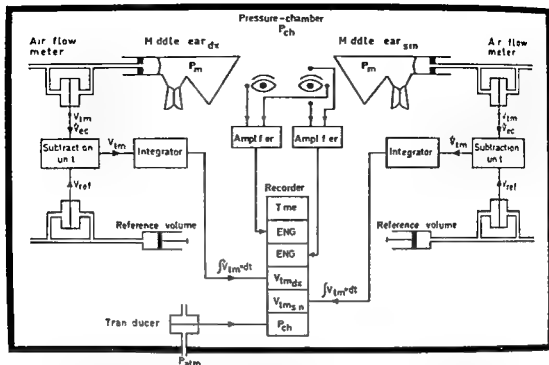


Fig. 1 Outline of the equipment used for recordings of the displacement of the eardrums ( $V_{tm}$ ) at changing ambient pressure ( $P_{ch}$ ). The equipment as well as the sub-

jects are placed in a pressure chamber. For further details see text and list of symbols.

increased indirectly by lowering the ambient pressure (simulated ascent) and the subjects were instructed to avoid active equilibration. A pressure chamber was used in which it is possible to simulate ascents of 90 cmH<sub>2</sub>O in 25 sec, with a constant rate of pressure decrease (4 cmH<sub>2</sub>O/sec) during 90% of the time. The induced relative overpressure in the middle ear caused an outward displacement of the eardrum. A method was used (Ingelstedt et al., 1967, Elner et al., 1971) by which it is possible to record the volume displacement of the eardrum ( $V_{tm}$ ) in relation to its neutral position at changing ambient ( $P_{ch}$ ) or middle ear ( $P_m$ ) pressure.

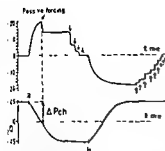
A polyethylene catheter with its end passing through a rubber disc was inserted into the inner bony part of the external ear canal airtightly connecting the space between the eardrum and the rubber disc with the measuring device. There was a free air communication between the outside of the eardrum and the ambient pressure, and the airflow caused by the eardrum displacement ( $V_{tm}$ ) was recorded

by means of a flowmeter. The flow  $V_{fm}$  can be eliminated for an isolated recording of  $V_{tm}$  during ambient pressure changes. This elimination is possible by the use of an identical flowmeter system adjusting a reference volume identical with the ear canal volume. Then the flow signal ( $V_{fm}$ ) is integrated and  $V_{tm}$  is recorded. Fig. 2 gives an example of a recording.

From these recordings of  $V_{tm}$  and  $P_{ch}$  it is possible to calculate the pressure gradient across the eardrum ( $P_{tm}$ ), i.e. the relative overpressure in the middle ear, at the moment when the onset of the vestibular response (nystagmus) was recorded. These calculations were made as described by Ingelstedt et al. (1974). For further details of equipment and method see Ingelstedt et al. (1967) and Elner et al. (1971).

## II Pressure gradient across an intact eardrum by change of pressure in the external ear canal

The pressure in the external ear canal ( $P_{ec}$ ) on the one side was lowered with a pressure



Recording of eardrum volume displacement ( $V_{tm}$ ) and chamber pressure ( $P_{ch}$  in  $\text{cmH}_2\text{O}$ ). Eardrum movement outwards (+) and inwards (-). Each examination starts with a simulated descent with a pressure of  $+45 \text{ cmH}_2\text{O}$ . It is then checked that the ear is in its neutral position ( $V_{tm} = 0$ ) after active ventilation. The simulated ascent then starts (a) with a decrease of  $90 \text{ cmH}_2\text{O}$ . The Eustachian tube moves passively (passive forcing), and the eardrum moves inwards to the neutral position when the middle ear pressure is equilibrated to the chamber pressure by ventilations (arrows). When the chamber pressure is increased (b) the eardrum moves outwards and when the ear pressure is equilibrated by deglutitions (arrows) the eardrum again moves to its neutral position.

is fully connected to the external ear canal as shown in Fig 3. By this method it was possible to induce an outward displacement of the eardrum as mentioned above.

#### Underpressure in the middle ear with pressure gradient across the eardrum and an immobile eardrum

With the equipment shown in Fig 3, the middle ear pressure ( $P_m$ ) was increased directly via free communication between the middle ear and the external ear canal through a transmyringeal tube (inner diameter  $0.8 \text{ mm}$ ) (Fig 3b). During investigations eye-movements were recorded simultaneously with the pressure recordings by means of electronystagmography (ENG), and recordings were performed in total darkness with the subject's eyes open (Tjernström, 1973). Flow-volume measuring unit and the pressure-measuring unit (cf Figs 1 and 3) were calibrated before and after every examination according to the original method. Eye-movements were calibrated for  $30^\circ$  in each direction before and after every test.

## EXPERIMENTAL PROCEDURE

### A Before myringotomy

(1) The subjects were exposed to simulated ascents in the pressure chamber and the volume displacement of the eardrum ( $V_{tm}$ ) was recorded during passive forcing of the Eustachian tubes from both sides, together with ENG-recording of eye-movements. The subjects were examined while seated and were instructed to keep their eyes open in total darkness and to report onset of vertigo.

(2) Underpressure was induced locally in the external ear canal with the equipment shown in Fig 3. The pressure was controlled so as to cause an eardrum movement outwards of the same degree as that caused in the experiments described above (procedure A 1).

### B After myringotomy and application of transmyringeal tube

Myringotomy was performed on the side with the highest forcing pressure, which was found to be on the right-hand side in both subjects.

(1) The subjects were exposed to simulated ascents and passive forcing of the left Eustachian tube, while the pressure in the right middle

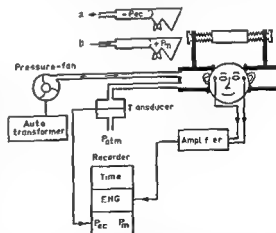


Fig 3 Outline of the equipment used to induce over- and underpressure in the external ear canal (a) Negative pressure in the external ear canal ( $P_{ec}$ ), (b) overpressure in the middle ear ( $P_m$ ) through a perforation in the eardrum. The pressure fan is connected into the external ear canal by a plastic cylinder airtightly fixed around the subject's external ear.

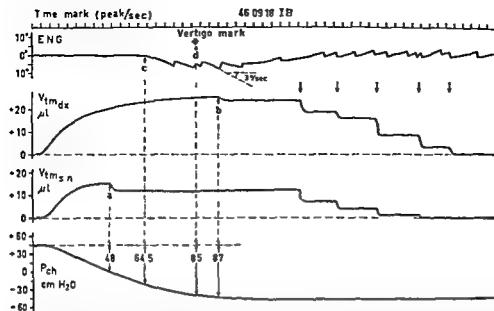


Fig 4 Electronystagmogram (ENG), volume displacements (in  $\mu\text{l}$ ) of right ( $V_{tmdx}$ ) and left ( $V_{tmdn}$ ) eardrums and chamber pressure ( $P_{ch}$  in  $\text{cmH}_2\text{O}$ ) during passive forcing of the Eustachian tubes. Arrows mark deglutitions. Passive clearing of the left ear (a) and right ear (b). Nystagmus starts when the ambient pressure changed by  $64.5 \text{ cmH}_2\text{O}$  (c)—slow nystagmus. The subject reports vertigo (d) when the chamber pressure is lowered by  $85 \text{ cmH}_2\text{O}$ . For more complete text.

ear followed the chamber pressure ( $P_m - P_{ch}$ ) owing to the transmyringal tube.

(2) With the equipment shown in Fig 3 overpressure was induced locally in the right middle ear via the transmyringal tube. The pressure level and the speed of the pressure change were made identical with those recorded in the experiments described above (procedure A 1).

## RESULTS

Figs 4, 5 and 6 are recordings taken from the different experimental situations on subject I. The examinations of the subject II showed compatible results.

### A Before myringotomy

(1) Simulated ascents with passive clearing of the ears elicited vertigo and nystagmus in both subjects. Fig 4 shows that the nystagmus started when the ambient pressure was lowered by  $64.5 \text{ cmH}_2\text{O}$ . The calculated relative overpressure in the right middle ear, i.e. the pressure gradient across the eardrum ( $P_{tmdx}$ ) was at that moment  $59.8 \text{ cmH}_2\text{O}$  (NPL = nystagmus pres-

sure level) and the pressure difference between the right and the left ear at the same time was  $42.5 \text{ cmH}_2\text{O}$ . The NPL for subject I was between  $59.8$  and  $64.1 \text{ cmH}_2\text{O}$ , and the pressure asymmetry between  $42.5$  and  $47.2 \text{ cmH}_2\text{O}$  (3 examinations). Corresponding data for subject II was NPL =  $54$  and asymmetry =  $\text{cmH}_2\text{O}$ .

(2) Underpressure locally in the right external ear canal ( $P_{ec}$ ) caused an eardrum movement outwards. The speed and the magnitude of the pressure decrease was controlled so as to cause an outward movement of the same degree as in the experiments with passive forcing of the tubes. The subjects reported no vertigo and no nystagmus was recorded.

### II After myringotomy and application of transmyringal tube

(1) The subjects were exposed to simulated ascents without active clearing of the ears. During the ascents a relative overpressure was built up only in the intact left ear causing a pressure asymmetry between the ears. As is seen in Fig 5 about the same asymmetry was

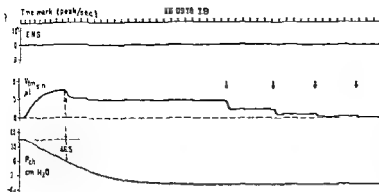


Fig 5 Electronystagmogram, volume displacement of left eardrum ( $V_{tm}$  in  $\mu$ l) and chamber pressure ( $P_{ch}$  in  $\text{cm H}_2\text{O}$ ) during passive forcing of the left Eustachian tube. A transmyringal plastic tube in the right ear prevented pressure gradients across the eardrum on that side.

as that recorded in the experiments with passive forcing of both Eustachian tubes (cf. Fig 4). The calculated forcing pressure (FPL = forcing pressure level),  $P_{tm}$  was 42.6  $\text{cm H}_2\text{O}$ . Despite the same asymmetry no vertigo appeared and no nystagmus was recorded. Thus asymmetry *per se* is not sufficient to cause vertigo.

(2) Overpressure was induced locally in the external ear canal of the ear with the transmyringal tube (right ear). Owing to this tube the pressure in the middle ear ( $P_m$ ) followed the external ear canal pressure without any displacement of the eardrum. The speed of the pressure change was controlled so as to be the same as that recorded at the passive forcing of the tubes with intact eardrums, and the pressure increase in the external ear canal was interrupted before the tube was forced open passively in order to avoid airflow through the middle ear, which could otherwise cause caloric stimulation. As is seen from Fig 6 it was possible to record nystagmus of about the same intensity

and starting at about the same pressure level as was seen in the experiments with passive clearing of both ears (cf. Fig 4). The subjects also reported vertigo of about the same intensity as that at the experiments with passive clearing of the ears.

## DISCUSSION

The results of the present study provide further evidence that a relative overpressure in the middle ear may stimulate the vestibular system. The overpressure was induced in two different ways, by lowering the ambient pressure (intact eardrum) or by overpressure induced locally via a transmyringal tube (direct to the middle ear). Roughly the same vestibular response was seen despite the different ways of inducing the relative overpressure, and a prerequisite for inner ear stimulation seems to be that the overpressure should reach a certain level. The pressure level is also found to be of a magnitude that

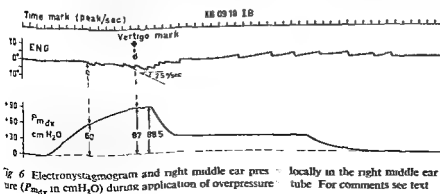


Fig 6 Electronystagmogram and right middle ear pressure ( $P_{mdx}$  in  $\text{cm H}_2\text{O}$ ) during application of overpressure locally in the right middle ear through a transmyringal tube. For comments see text.

might occur during flight and diving. It is seen from the present work that movements of the eardrum in connection with the ossicles *per se* do not affect the vestibular system. It is also seen that approximately the same vestibular stimulation was recorded under the two different experimental conditions with overpressure in the middle ear although the eardrum was immobilized by a transmyringal tube in one of the situations.

Pressure changes might affect the inner ear indirectly by temperature variations (caloric stimulation) caused by expansion or compression of the middle ear gas volume due to pressure variations in the middle ear ( $\Delta P_m$ ). To exclude the possibility of a caloric stimulation it is necessary to discuss the pressure changes in the middle ear in the various experimental situations.

In the experiments with simulated ascents and passive clearing of the ears (cf Fig 4) there is a great relative overpressure in the right middle ear ( $P_{\text{tm,ex}}$ ) before the Eustachian tube is forced open, and the inner ear stimulation is found to start when the relative overpressure has reached 59.8 cmH<sub>2</sub>O. The absolute pressure change in middle ear ( $\Delta P_m$ ) at this moment has then reached 4.9 cmH<sub>2</sub>O. This pressure change can be calculated by using Boyle's law according to Ingelstedt et al (1967 and 1974), and Andréasson (1973)

$$\Delta P_m = \frac{\Delta I_{\text{tm}} + \Delta I_{\text{muc}}}{I_m} P_{\text{md}}$$

The following factors are required for this calculation

- $\Delta I_{\text{tm}}$  - the recorded eardrum displacement outwards on the right hand side (in the example given in Fig. 4 subject 1 = 22.4  $\mu\text{l}$ )
- $\Delta I_{\text{muc}}$  - the calculated volume change of the middle ear mucosa (0.5  $\mu\text{l}$  cmH<sub>2</sub>O) (Andréasson et al, 1974) (in the example given in Fig. 4 subject 1 = 29.9  $\mu\text{l}$ )
- $I_m$  - the middle ear volume calculated by using a roentgenological planimetric method which means transferring the area of the air filled middle ear system, measured from X-ray films to a volume according to Andréasson (1973) (in subject 1 = 10 900  $\mu\text{l}$ )

In some experiments, overpressure was introduced locally in the middle ear through a transmyringal tube (cf Fig. 6). In a comparison of the experimental situations with an intact and a perforated eardrum approximately the same relative overpressure (59.8 resp. 60 cmH<sub>2</sub>O) was recorded at the moment nystagmus started (cf Figs. 4 and 6), but the absolute pressure change ( $\Delta P_m$ ) was different, 4.9 resp. 6.0 cmH<sub>2</sub>O. Approximately the same inner ear stimulation was, however, recorded. Exclusion of caloric stimulation in the above mentioned situations is further confirmed by the experiments in which a transmyringal tube was applied on one side. Then  $\Delta P_m$  during ascents follows  $\Delta P_{\text{tm}}$  with a range of 90 cmH<sub>2</sub>O. Despite this great relative pressure change no inner ear stimulation was recorded. Thus the vestibular stimulation does not seem to be caused by a pressure change.

Is it possible that the overpressure might affect the vestibular system directly via the oval and/or the round window by means of an ear fluid motion (endo- and perilymph) causing a cupula deviation? It has been shown by several authors many years ago, in sections of preparations from homo (e.g. Benli 1908, Nylen, 1923) and of living animals (Karak, 1935), that the labyrinthine pressure rises or falls according to pressure changes in the middle ear. Inner ear fluids might be set in motion by overpressure in the middle ear in different ways, for instance by a fluid displacement between the two windows of the cochlea type caused by acoustic vibrations. However, it is difficult to understand such a mechanism if an overpressure in the middle ear is equally on all resilient structures of the two windows. The pressure is, however, owing to the rigidity of the bony frame of the round window plate. From the present work it is seen that the pressure change in the middle ear is chiefly the round window, greater than that in the oval window, also possible.

stapes could even move the stapes against pressure gradient when the eardrum is displaced outwards, but the function of the ossicle chain in such circumstances is not exactly

Another factor responsible for inner ear fluid motion might be a fluid displacement via the lymphatic and perilymphatic ducts, but these differ as to the patency of these ducts (Karlfors, 1924, Waltner, 1948, Lempert et al., 1964, Allen, 1964, House, 1964, Ritter & Lawless, 1965, Anson, 1965, Holden & Schuknecht, 1968)

Finally, the vestibular system might be affected by a circulatory disturbance causing ischaemia due to venous or capillary stasis caused by the increased middle and inner ear pressure. A venous stasis together with a lymphatic stasis might also cause a further intralabyrinthine pressure increase interfering with circulation of the inner ear fluids. Blood vessel connections between the middle and the inner ear have been shown to exist (e.g. Hansen, 1971) and the pressure effect on the middle ear pressure might be transmitted to the inner ear via these connections.

In view of these various mechanisms which have been discussed, the above-mentioned work by Lyden & Karlfors (1921) is of particular interest. These authors could show that a constant overpressure in the middle ear, in subcutaneous space with an eardrum perforation, caused a stimulus which gradually increased in strength during 10–20 seconds and then ceased. In their opinion the mechanism is an "impression on the holding portions of the labyrinth, which could cause a lymphatic movement with accompanying irritation."

## ZUSAMMENFASSUNG

Ein absoluter Überdruck wurde mit freier Luftzufuhr in den Gehörgang im Mittelohr hervorgerufen und in seiner Wirkung mit relativem Überdruck gleicher Stärke verglichen. Der relative Überdruck wurde indirekt durch Senkung des Umgebungsdruckes erzeugt. Dieselbe vestibuläre Reaktion konnte mit den genannten unterschiedlichen Methoden hervorgerufen werden unter Voraussetzung, dass der Überdruck

ein gewisses Mindestmass erreichte. Ein gewisser Druckunterschied zwischen beiden Mittelohren ist an der Wirkung auf das Vestibularorgan mitbeteiligt. Der Überdruck übt seine Wirkung ohne Anteilnahme des Trommelfells aus, blosser Bewegungen des Trommelfells hatten keinen Einfluss auf das Innenohr. Die Möglichkeit eines kalorischen Reizes hervorgerufen durch Temperaturschwankungen im Gasvolumen des Mittelohres wurde beachtet und konnte ausgeschlossen werden.

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## INVESTIGATION OF POSITIONAL AND POSITIONING NYSTAGMUS BY USING A NEW TYPE OF ELECTRICALLY-DRIVEN ROTATION TABLE

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(Received February 20, 1974)

The different methods for investigating positional and positioning nystagmus are presented and discussed. The latest results concerning the interaction between the utricle and the horizontal semicircular canals are mentioned and based on these findings, a new electrically-driven rotation table for investigating positional and positioning nystagmus is demonstrated. The mode of operation and the predictable, normal results are presented.

Investigation of positional nystagmus can be carried out in several different ways, with the patient in either a sitting or a supine position. Usually the investigation is performed in two different planes

the sagittal plane, sitting, supine or with the head hanging out over the upper edge of the table

The frontal plane, right or left lateral position with supine position as the starting position

A special kind of positional nystagmus is the so-called positioning nystagmus, i.e. nystagmus immediately after taking a certain position. Zee (1955) recommends a rapid alteration of position in the sagittal plane, from sitting to supine, with head hanging and back again to a sitting position. By this means a transitory, usually rotatory or horizontal-rotatory nystagmus is obtained, which lasts between a few seconds and 1½ min. The nystagmus is usually reproducible. Another procedure is used by Hallpike (1952) who within 3 sec changed patient's position

sitting to supine with the head simultaneously turned 45° to either side. This, as a rule, induces a rotatory nystagmus of short duration.

However, the way in which these tests are performed always involves a risk of interference from other sensory organs. Turning the head in relation to the shoulders also stimulates the so-called neck reflexes. Therefore Nylen (1950) recommended that head and body should be moved together. However, the importance of this was denied by Cawthorne (1954).

The movement factor is also of great importance. In order to be able to term the findings positional nystagmus, it is necessary to exclude every kind of interference from the semicircular canals, which may be stimulated if the angular moment is too large.

Attempts have been made to eliminate these false sources by constructing special posture-tables. Such mechanically-driven tables have been constructed by Quix (1926), Grahe (1927) and Fromm & Nylen (1935). Though this has certainly eliminated the problem of neck reflexes the manual drift of the table has not been controllable or reproducible. Consequently, Aschan et al. (1956) constructed an electrically-driven posture table, which could accelerate by 0.05-0.1°/sec<sup>2</sup> up to a speed of 1-3°/sec. Although this type of table has made it possible to make interesting studies on positional nystagmus, owing to its limited possibilities, it cannot be used for investigations concerning

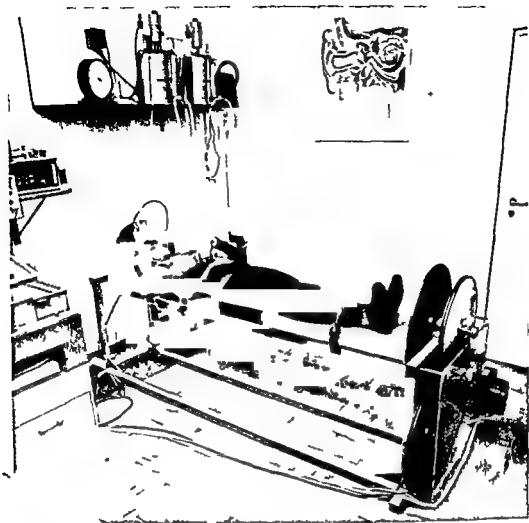
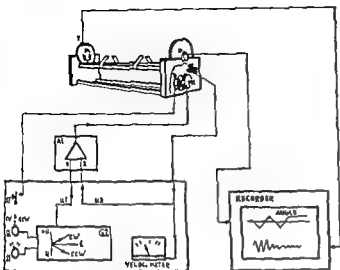


Fig. 1 The rotation table

used for systematic studies on the interaction of utricular and semicircular-canal activities. As the function of the otolith organs was not known in detail in the mid 1950s, especially regarding the oculomotion, the findings reported were of limited value. However, investigations made during recent years have thrown new light on this problem. Fluor & Mellstrom (1970) have shown that electrical stimulation of different areas on the utricular surface induces distinct eye movements in varying directions depending on the area stimulated. Subsequently, Fluor & Siegborn (1973a, b, c) in the cat and Fluor (1973) in man have shown that these different areas cooperate with the semicircular canals in an intimate and systematic way in their influence on the oculomotor system. The results show that

the horizontal nystagmus obtained after a lateral labyrinthectomy is facilitated if the patient or the animal is tilted around his or her longitudinal axis towards the diseased ear, and is inhibited if the tilt is towards the sound ear. From these experiments it is concluded that the utricle and the ipsilateral horizontal semicircular canal function synergistically, whereas the utricle and the contralateral horizontal semicircular canal function antagonistically. Therefore, a right beating nystagmus becomes more pronounced in a left lateral position than in a right

The above-mentioned results can now be used clinically to investigate positional and positional nystagmus as an expression of the interaction between the otolith organs and the semicircular canals and especially between the utricle



- |                      |                               |
|----------------------|-------------------------------|
| A1 Amplifier         | Z Connection for electrodes   |
| B Brake              | G1 Acc/Dec value switch       |
| G1 Tachogenerator    | G2 CW/CCW acceleration switch |
| G2 Regenerator       | S3 Brake switch               |
| M1 Table drive motor |                               |

Fig 2 Schematic picture of the electronic equipment

and the horizontal semicircular canals. The reason why the horizontal semicircular canals have been chosen is partly because a modulation of the utricular function as the vertical or rotatory nystagmus, and partly because horizontal nystagmus is the easiest to record electronystagmographically.

### THE ROTATION TABLE MSA<sup>1</sup>

In order to make these investigations possible we have constructed in collaboration with MSA (Stille-Werner AB, Stockholm, Sweden) an electrically-driven rotation table with wide variability in the stimulation pattern.

To a steel stand is attached a bunk  $2 \times 0.5$  m, which can be rotated around a horizontal longitudinal axis. The subject's head can be placed in a head holder provided with two occipital and two temporofrontal pads, the latter are attached to the head by means of a screw so that it is firmly fixed. The body is fixed to the bunk partly by two lateral thoracic supports,

and partly by four safety belts around the body and the bunk (Fig 1).

In order to ensure that the table functions without causing vibrations and other disturbances it is driven by an electrically controlled servosystem, which operates as follows (Fig 2). The angular velocity of the driving motor M1 is determined by the voltage U1, which represents the value of the desired velocity. U1 is fed into input 1 of the amplifier A1. The angular velocity of the driving motor is measured by the tachogenerator G1, which produces a voltage output U2 proportional to the angular velocity of the driving motor. Thus, U2 represents the value of the actual velocity. U2 is fed into input 2 of the amplifier A1, where it is compared with the control voltage U1. If there is a difference between the actual velocity and the desired velocity, there will of course be a corresponding difference between U2 and U1. The amplifier will then produce an output signal which will change the angular velocity of the motor until U2 equals U1.

If the control voltage U1 is increased linearly with time, a constant acceleration of the

<sup>1</sup> MSA = Medicinska Segurhetsföretag AB

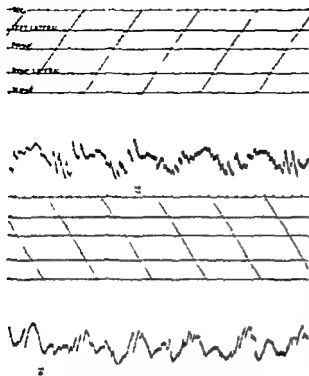


Fig. 3 Electronystagmographic recordings from a normal person during constant rotation at a speed of 60°/sec around the longitudinal axis. The upper half of the curves indicates the rotation of the table; the lower half indicates the horizontal nystagmus. The upper curves show nystagmus towards the right during clockwise rotation, inhibited in the right lateral position and facilitated in the left position. The lower curves show nystagmus to the left during counterclockwise rotation, inhibited in the left lateral position and facilitated in the right lateral position.

of the table is obtained. If  $U1$  is decreased linearly with time, constant deceleration is obtained.

$U1$  is generated in the ramp generator  $G2$ , which can be set to deliver the desired change in voltage per unit time. At any time desired, switch 2 can be used to set the ramp generator so that the voltage-change process is stopped. As  $U1$  then becomes constant, the motion of the table will stop accelerating and constant angular velocity will be maintained.

The table can be brought to a standstill by controlled deceleration or by activation of the brake  $B$  by the switch  $S3$ . When the brake is actuated, the voltage from the ramp generator automatically falls to zero and consequently the drive motor is stopped.

## Performance

1. The table can be accelerated or decelerated from 0 to 20°/sec<sup>2</sup> both clockwise and counterclockwise.

2. It can be rotated at constant speed from 0 to 100°/sec.

3. It can be stopped instantly in every position.

4. It can be coupled free and turned around manually if in certain initial or basic position desired.

## Recording

The movements of the table are recorded by potentiometer  $P$ , situated on the rotation axis, which indicates both motion of the table and its angular position.

The eye movements are recorded electromographically with electrodes for horizontal nystagmus, caudal to both lateral canthi, in order to prevent artefacts due to movements from the temporo-frontal head pads. However, it is appropriate with a warning. In the supine position the utricles are depressing the eyes, which has a negative effect on the horizontal nystagmus. Therefore the patient is told to look straight forward during the recording. The third electrode is placed in the middle of the forehead. The signals from the electrodes are conveyed to the terminal strip  $T$  and pass via slip rings to the recorder.

## Mode of stimulation

To investigate positional nystagmus, the patient is rotated at constant speed 0.1–0.5 sec into the supine position to the right or left lateral position, in which the table is stopped for 1 min.

To investigate positional nystagmus, the patient is accelerated clockwise or counterclockwise at a speed of 0.1–0.5 sec. During this period, in a postacceleratory period, which lasts 1 min, the head is in a position of 45° to the right or left. The reason for this is

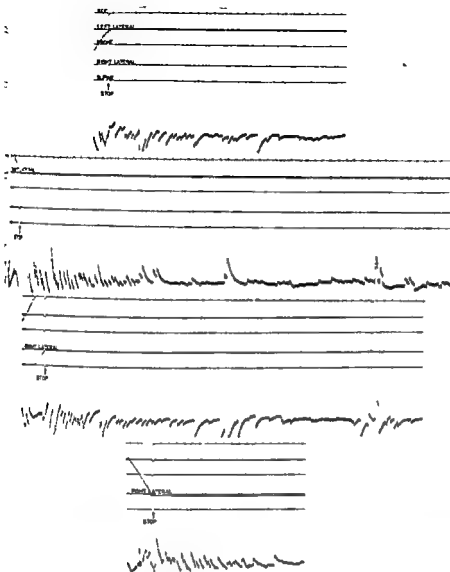


Fig. 4. Electronystagmographic recording of postrotatory horizontal nystagmus from a normal person when rotation at a speed of 60/sec is suddenly stopped in left lateral position (curves 1 and 2) or right lateral position (curves 3 and 4) lateral position (curves 1 and 3) or counterclockwise (curves 2 and 4) rotation. The upper half of one curve

shows the position of the table, the lower half the direction and intensity of nystagmus. In the positions where the utricles and the semicircular canals are functioning synergistically the duration of postrotatory nystagmus is much longer—about twice—than in the positions where they are functioning antagonistically.

here the right beating nystagmus, induced by clockwise acceleration, is facilitated by the right utricular input, i.e. in the left lateral position, there is a distinct increase in intensity of the nystagmus, whereas in the right lateral position, there is a distinct decrease in intensity. Here the increased activity from the left utricle is functioning antagonistically to the right hori-

zontal semicircular canal, an inhibition of nystagmus is often observed (Fig. 3). The table is then suddenly stopped in the left lateral position and the duration and intensity of the postrotatory left-beating nystagmus is studied. Because the increased activity of the right utricle in this position functions antagonistically to the in-

creased activity of the left horizontal semicircular canal, the duration of the nystagmus will be short (Fig 4)

A few minutes later the patient is accelerated counterclockwise in the same way as earlier. The left-beating nystagmus, which the patient most often has even during constant speed, is then studied. Here an increased intensity of nystagmus is observed in right lateral position, which is due to synergism between the increased inputs from the left utricle and the left horizontal semicircular canal. In the left lateral position nystagmus is inhibited due to antagonism between the increased activities of the right utricle and the left horizontal semicircular canal. The table is then stopped, also this time in the left lateral position, and the duration of the postrotatory right beating nystagmus is recorded. Here the right utricle functions synergistically with the increased input from the right horizontal semicircular canal. Therefore, the duration of the nystagmus becomes considerably longer than that induced by the former stimulation (Fig 4), despite the patient's position being the same in both situations.

The investigation is then continued again with clockwise acceleration, constant speed and sudden stop, but this time in the right lateral position. The left beating postrotatory nystagmus is here prolonged by facilitation due to synergism in input from the left utricle and the left horizontal semicircular canal (Fig 4). Finally, the patient is accelerated counterclockwise, the variations in nystagmus during constant speed are studied and the table is stopped again in the right lateral position. The right-beating postrotatory nystagmus due to increased input from the right horizontal semicircular canal is inhibited here by the antagonistically operative increased activity of the left utricle, and the duration of the nystagmus is shorter than formerly in the same position (Fig 4).

## ZUSAMMENFASSUNG

Die verschiedenen Methoden für die Untersuchung von Lage- und Lagerungsnystagmus sind dargestellt und diskutiert worden. Die letzten Resultate der Zusammen-

arbeit zwischen Utriculus und den horizontalen Kanälen werden nachgewiesen, und auf Grund der Resultate wird ein neuer, elektrisch getriebener Testonstisch für die Untersuchung von Lage- und Lagerungsnystagmus demonstriert. Die Art der Symptome und die erwarteten normalen Ergebnisse werden besprochen.

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## OPTOKINETIC-GRAVICEPTIVE INTERACTION IN DIFFERENT HEAD POSITIONS

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(Received February 25, 1974)

**Abstract** With increasing lateral tilt of the head ( $0^\circ$ ,  $30^\circ$ ,  $45^\circ$ ,  $60^\circ$ ) the apparent vertical (AV) is progressively inclined in the same direction. Simultaneously, the variance of settings of a test edge increases, consistent with the theory of a progressive decline in the effectiveness of the statolith organs. The AV can also be influenced by moving visual scenes rotating around the observer's line of sight. It has been argued that visual motion information, which also results in apparent body tilt, contributes to the central computation of orientation of the gravitational vector. If with lateral head tilt graviceptive sensory input is less accurate and less powerful the visual influence should become more potent. Indeed, displacements of AV induced by exclusive visual stimulation with the head erect are moderate but increase progressively with larger head tilt angles. The potentiation of the net visual effect on AV in an inclined head position is maximal if the visual stimulus is moving opposite to the head tilt. The demonstrated visual graviceptive interaction seems to have functional implications for postural orientation.

Gravitational orientation depends on two major sources of experience, the exteroceptive visual and the proprioceptive postural. The latter is mainly provided by graviceptive information from the vestibular maculae but also from somesthetic gravireceptors in the trunk. Although orientation depends on these distinct sources of information, we do not have in our experience distinct conceptions of the upright, one visual and the other postural. Our experience of spatial orientation is unitary. The final processes to which both visual and postural factors contribute produce the experience of a single upright (Asch & Witkin, 1948a).

This work was supported by the Deutsche Forschungsgemeinschaft, SFB 70 (Hirnforschung und Sinnesphysiologie).

The effects of the observer's visible surroundings on his spatial orientation have been extensively studied. Most investigations have been concerned with the effects of steadily tilted contours or panoramas on the apparent visual and postural vertical. Although controversial in some respects, the results generally agree that the apparent vertical is moderately displaced in the direction of tilt of the visual scene (literature reviewed by Howard & Templeton, 1966). It has been reported that a visual surround continuously rotating around the observer's line of sight induced much stronger tilts of the apparent upright ranging up to  $40^\circ$  (Dichgans et al., 1972) and affects body posture (Brandt et al., 1973). Since the moving visual display did not entail cues to visual orientation that would conflict with those of gravity it was concluded that motion as such caused the perceived tilt. The fact that the tilt was common to both visual and postural orientation led to the hypothesis that the shift occurs in the internal representation of the gravity vector. It was assumed that the subjective orientation of the gravity vector is centrally computed from inputs originating in gravireceptors and the retina (Dichgans et al., 1972). The limitation of visually induced tilt was thought to be due to conflicting information given by excitation of the otolith and graviceptive pressure receptors.

Indications of a different effectiveness of statolith organs in different head position



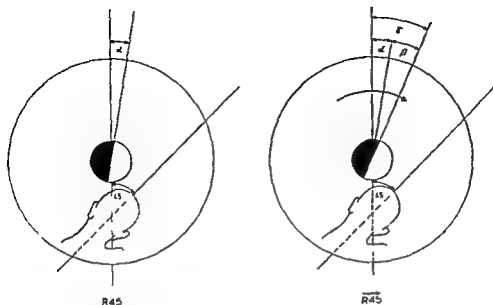


Fig. 1 Schematic drawing of the different angles measured. On the left head inclination only. On the right

with additional rotation of the visual display. For further explanation see text.

Colenbrander, 1963/64, Graybiel & Clark, 1962, Graybiel & Patterson, 1955, Hofman, 1910, Nagel, 1898, Quix & Eysvogel, 1929, Schöne, 1964) found signs of increase in variance of graviceptive information with increase of body tilt. Others, more specifically, demonstrated a decrease in significance of statolith inputs concomitant with an increase in significance of visual cues for orientation of the apparent vertical when the head was progressively inclined and steadily tilted lines were presented at the same time (Asch & Witkin, 1948b, Bischof & Scheerer, 1970, Schöne & Udo de Haes, 1971). Young (1971) assumed a 'confidence factor' for weighing statolith information at different head positions and consequently a changing nature of visual-vestibular interaction in perception of orientation.

Young's hypothesis was tested and confirmed in experiments where the subject viewed a large visual pattern rotating around his line of sight while his head was held in different positions. The subjects of Young et al. (1974) reported that the visually induced tilt effect increased markedly for the head tilted laterally 90° or inverted. These observations were confirmed in the series of experiments presented in this

paper by measuring the magnitude of induced tilt obtained when the head was upright or tilted laterally 30, 45 or 60°, respectively. The results not only confirm the assumption of a different weighting coefficient for graviceptive information depending on the head position in space but also its dependence on the directions of body head inclination and movement of the visual display.

## METHODS

### The experimental apparatus

A large display that could be rotated about the observer's line of sight was mounted in front of the subject. Its surface was covered with a coloured floral pattern that by itself entailed no cues to orientation. The disk, subtending 18° of visual angle, was viewed binocularly. The rest of the visual field was masked off with rings of dark cardboard. The shaft mounted at the centre of the disk was connected to a drive motor. As in earlier experiments (Dichgans et al., 1974; Held et al., in press) a smaller disk—referred to as the target disk—was mounted on a concentric shaft just in front of the large disk. The target disk subtended 18° of visual angle and carried a straight edge that divided its surface

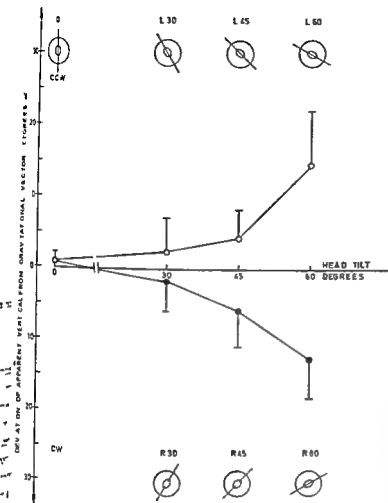


Fig 2 Deviation of apparent vertical (AV) from gravitational vector at different head positions (x) to the left (upper half) and to the right (lower half of the graph) Means and standard deviations

into black and white semicircular areas and a fixation point at the midpoint of the edge. The orientation of this edge could be controlled by the observer by turning a potentiometer that in turn controlled the position of a servo-motor connected to the shaft of the target disk. The shaft was also connected to a second potentiometer which linearly varied a voltage recorded on a strip chart. The resolution of the measurements thus taken was  $1^\circ$ .

Each subject was seated with the midpoint between his eyes aligned with the axis of the visual display. His head was rigidly attached to a head holder by means of a bite board and by belts fastened around his occiput. The orientation of the head could be altered by tilting the head holder about the sagittal axis. In every

new position the accuracy of alignment of the subject's line of sight with the display axis was controlled and corrected if necessary.

### The experimental procedure

The orientation of apparent vertical (AV) with respect to gravity was repeatedly measured in 3 experienced subjects with the head erect and the head inclined  $30^\circ$ ,  $45^\circ$  or  $60^\circ$  towards the right (R) or left (L) shoulder. At every head position measurements were alternatively taken with the visual stimulus stationary ( $N=20$ , in each subject) or moving at  $60^\circ/\text{sec}$  clockwise ( $\rightarrow$ ) or counterclockwise ( $\leftarrow$ ) ( $N=10$ ).

The sequence of head tilt angles and directions of disk rotation was in random order. The r

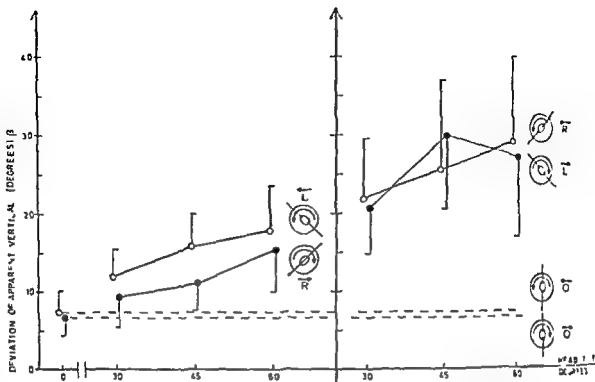


Fig. 3. Additional deviation of apparent vertical induced by the rotating visual display ( $\beta$ ) at different angles of head inclination (abscissa). Disk rotation and head inclination towards the same direction (left graph) and in opposite directions (right graph).  $\circ$ , AV to the left;  $\bullet$ , AV to the right.

The dashed lines depict the deviation with the head erect and only the disk moving to the left and right respectively. Means and standard deviations.

ing tilt angles of AV with respect to gravity were called  $\alpha$  (Aubert or Muller phenomenon) if the visual stimulus was stationary and  $\gamma$  if the disk was moving. The net tilt effects induced by the moving stimulus were then calculated as  $\beta$  by subtracting  $\alpha$  from  $\gamma$  (Fig. 1). Means and standard deviations were determined for each of the three angles in each of the 7 head positions. Means of angles were calculated after putting a negative sign to every tilt of AV that was opposite to the head tilt (Muller phenomenon). These experiments followed a pilot study that yielded consistent results. In this study 40 inexperienced students had been tested with the head erect and tilted 45° to either shoulder. But in contrast to the main experiment, reported here, measurements with the head tilted were taken only once and with one head tilt direction from each subject thus providing independent samples (Diener, thesis, in preparation).

## RESULTS

### Deviations of apparent vertical from or of gravity in different

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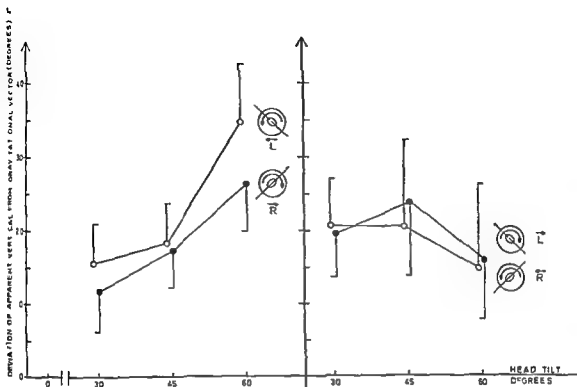


Fig. 4. Total tilt effects ( $=\alpha+\beta$ ) with the visual stimulus moving and the head at different angles of inclination. Disk rotation and head inclination towards the same

direction (left graph) and in opposite direction (right graph). ● AV to the right, O AV to the left.

vertical. According to Nagel (1898), Bourdon (1906), Müller (1916) and Bauermeister (1964), the Müller phenomenon persists until the head tilt angle exceeds  $60^\circ$ . In the three subjects participating in this study we have not seen the Müller phenomenon when the head inclination reached  $60^\circ$ . The accuracy of settings to AV with the head upright ranges within  $\pm 1.5^\circ$ , but decreases with increasing head tilts.

#### Tilt effects on apparent vertical induced by seen motion in different head positions ( $\beta$ )

The effects of a visual display rotating around the observer's line of sight increase with progressive inclination of the head. The increase can be seen in Fig. 3 (ordinate). It is represented by the deviation of the four curves from the dashed lines. The latter depict the tilt effect induced by the moving display in the CW- and CCW direction, while the head was erect. The

curves represent the data obtained when the head was laterally inclined. As can be seen from the picture, this increase depends on the directions of head tilt and display rotation. The average effects are overtly smaller when the two stimuli point towards the same direction than vice versa. This asymmetry cannot be explained as the result of summation of the "head" and "disk effect" since the data depicted in this graph have been obtained by subtracting the head tilt-effect ( $\alpha$ ) from the total effect ( $\gamma$ ) shown in Fig. 4. Fig. 3 therefore depicts the net effect of the moving visual display in different head positions. Standard deviations of "head effects" ( $\alpha$ ) and "visual effects" ( $\beta$ ) are very similar as long as the two point towards the same direction, but increase markedly when the visual stimulus moves opposite to the head tilt. As can be seen by comparing Fig. 2 and 3, the addition of the moving visual display ( $\beta$ )

the pure head tilt effect ( $\alpha$ ). Thus within the range of head inclinations explored the orientation of AV was always determined by the direction of the rotating visual display (Fig. 4).

## DISCUSSION

The data presented confirm the hypothesis that visual motion information and graviceptive information are centrally combined for the computation of the apparent orientation of gravity. Our argument is based on the finding that the effect of a visual display rotating around the observer's line of sight increases with progressive lateral inclination of the head, positions in which statolithic information is known to decline in accuracy (Colenbrander, 1963/64, Graybiel & Clark, 1962, Graybiel & Patterson, 1955, Quix & Eijssvogel, 1929, Schone, 1964) and "sensory weight" as one might like to call it. Our results seem to bear out that this decrease at least in part may be compensated by visual afference. The data may to some extent be contaminated by the fact that our subjects were tested with the head bent with respect to the body therefore allowing for possible interfering afference from joint receptors and graviceptive information from somatoreceptors from the trunk which was kept in the upright position. This interference, however, would only change the amount of induced tilt and is not conditional on the described phenomenon as was indicated by pilot experiments performed with the entire body tilted laterally. Earlier studies comparing the effects of body versus head inclination and head versus body inclination on AV yielded controversial results. Fischer (1927) found in one subject that the effects of head and trunk inclination are quite similar and add up to the equivalent of the effect of inclination of the entire body. Witkin & Asch (1948), Schöne & Udo de Haes (1968) and Wade (1968), however, found little difference between exclusive head tilt and tilt of the entire body.

The neurophysiological basis for this type of visual-vestibular interaction is not known. An interaction of graviceptive and visual inputs in

the vestibular nuclei, however, is likely since related interactions have been shown in animals with rotation in the horizontal plane about an earth vertical axis. Optokinetic information was shown to influence the discharge of the first order vestibular neurone in the goldfish (Klinke & Schmidt, 1970) and of the vestibular nuclei in the goldfish (Dichgans et al., 1973), rabbit (Dichgans & Brandt, 1972) and monkey (Henn et al., submitted). The functional importance of vestibular interaction during motion in the horizontal plane is rather obvious in that vestibular semicircular canals only provide information about changing velocities while the visual input contributes supplementary information about constant velocity (Brandt 1973, Dichgans et al., 1973). With motion about an axis that is perpendicular to orientation of gravity visual afferents may only interfere with canal input but also afference from statoliths and other graviceptive receptors. The interaction might not at all not exclusively occur at the level of the vestibular nuclei but at higher levels of the central nervous system. To clarify these mechanisms is a challenging subject for further investigation.

The functional importance of visual-vestibular interaction during motion in a vertical plane is not as well understood at the present time. It is conceivable, however, that the effect of relative motion of the visual environment caused by body displacement from real upright position corroborate the actual information from graviceptive receptors for postural adjustment. On the other line one might give a functional interpretation for the asymmetry in the amount of tilt induced by the moving visual display when the inclination and visual movement point in the same direction as opposed to the case when they are in opposite directions. Under natural conditions body tilt causes relative motion of the visual scene that is opposite in direction to the body displacement. Therefore, in this case, will corroborate with the actual information from graviceptive receptors for postural adjustment.

whereas with the two motions going in the direction visual afferents would antagonize tural uprighing process induced by gravi e inputs. The latter obviously represents tificial situation created in the laboratory s of no behavioural significance in normal A similar asymmetry has been found for ffects of steadily tilted contours which also onger when head tilt and contour tilt are osite directions (Mann, 1952, Witkin & 1948). It seems to us that the postural . of stationary and moving scenes repre respectively, the static and dynamic con ons of vision to graviceptive orientation

## ZUSAMMENFASSUNG

nehmender seitlicher Kopfneigung (0, 30, 45° sche ot die subjektive Vertikale zunehmend zur Seite gene gt. Gleichzeitig steigt die Varianz von ungen der visuellen Vertikalen an was durch nehmende Ungenauigkeit des Statolitheneinflusses ichter Kopfneigung erklärt werden kann. Die lenwahrnehmung wird auch durch großflächue

Bestimmung der Schwerkraftorientierung bei ut zunehmender Kopfneigung der graviceptive enger genau und gewichtet wird kann der Anteil entsprechend zunehmen. Tatsächlich sind ell induzierten Auslenkungen der wahrgenomme tiken bei aufrechtem Kopf geringer und nehmen ehmeender Kopfneigung zu. Die Potenzierung des uellen Einflusses auf die subjektive Vertikale bei igung ist am größten wenn der visuelle Bewe z in Gegenrichtung der Kopfneigung rotiert. Die ilte visuo-graviceptive Interaktion hat auch unter en Bedingungen eine funktionelle Bedeutung. Regulierung der Körperhaltung

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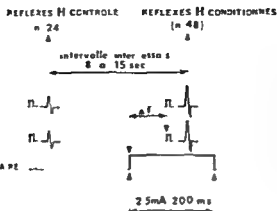
$\Delta t = 10, 20, 50, 100, 150 \text{ ou } 350 \text{ ms}$ 

Fig 1 Organisation de la double action stimulation vestibulaire (St V) stimulation de Hoffmann (H). Dans la série expérimentale, on applique d'un même côté (à gauche ou à droite) la stimulation vestibulaire appliquée simultanément sur les deux jambes. On distingue les réflexes H contrôlés (n=24 par série) des réflexes H conditionnés par la St V (n=48 par série). Commentaires dans le texte.

ficacité de la St V dépend du côté où elle est appliquée (à gauche ou à droite). Par ailleurs, nous soulignons le rôle de la latéralisation motrice des sujets dans la réactivité réflexe spinale: la facilitation des réflexes H dépend du côté de la réception électromyographique (jambe gauche ou jambe droite).

Les variations différentielles des réflexes enregistrés bilatéralement à la suite d'une St V monaurale sont donc affectées.

1 Par l'organisation asymétrique des voies vestibulo-spinales issues d'un labyrinthe (prépondérance des voies ipsilatérales)

2 Par la sensibilité différentielle des récepteurs labyrinthiques symétriques à la stimulation électrique (prévalence vestibulaire)

3 Par la réactivité différentielle des noyaux moteurs spinaux symétriques (latéralisation motrice)

## METHODES ET TECHNIQUES

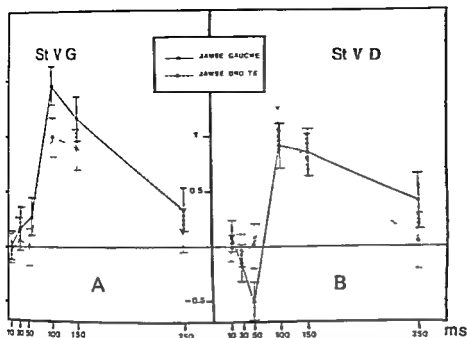
Vingt sujets normaux, étudiants de 18 à 25 ans, rémunérés et 2 sujets delabyrinthiques ont été examinés. Pour l'étude des effets spinaux de la St V, on enregistre le réflexe H dans un muscle extenseur du pied. Le sujet est assis dans un fauteuil spécialement équipé.

La stimulation réflexogène est une stimulation électrique percutanée du nerf sciatique poplité interne, appliquée selon la méthode définie par Paillard (1955): elle est distribuée simultanément

dans les deux jambes. On recueille la réponse réflexe H par électrodes de surface et mesure son amplitude au voltmètre électrique. Nous déterminons pour chacun des sujets l'amplitude maximale des réflexes H à droite et à gauche et nous réglons l'intensité de la stimulation réflexogène de façon à obtenir chaque jambe un réflexe d'amplitude maximale.

La St V est une stimulation électrique percutanée de la région otique. Elle consiste en un stimulus rectangulaire de 200 ms appliqué par un moyen d'un casque léger supportant deux électrodes d'argent: une anode de 2 cm de diamètre est appliquée en avant du tragus et une cathode de 1 cm de diamètre sur l'apophyse mastoïde située du même côté de la tête. La St V provoque chez le sujet assis des latrations de la tête, dans les conditions de stimulation elle est ajustée à une intensité subliminaire pour ces déplacements (soit 25 dB en moyenne).

Chaque sujet est soumis à 4 séries expérimentales réflexologiques: 2 avec la stimulation vestibulaire appliquée au côté gauche (St V G) et 2 avec la stimulation vestibulaire appliquée au côté droit (St V D). Chaque série (Fig 1) comporte 72 réflexes H dont 24 réflexes H contrôlés par la St V et 48 réflexes H conditionnés par la St V. Les réflexes H conditionnés sont provoqués à des délais  $\Delta t$  variables (10, 30, 50, 100, 150, 350 ms) distribués de façon



Modulation de l'amplitude des réponses réflexes d'origine par la stimulation vestibulaire électrique (1). Résultats moyens observés sur une population de sujets (A) Effets spinaux de la St. V appliquée au côté gauche (St. V G) (B) Effets spinaux de la St. V appliquée au côté droit (St. V D). En abscisses : temps en millisecondes entre le début de la St. V et l'application de la stimulation réflexogène. En ordonnées : amplitude des réflexes H exprimée en valeurs réduites (notes Z). Les

variations d'amplitude des réflexes H recueillis sur la jambe gauche (trait plein) et sur la jambe droite (trait pointillé) sont fonction du côté d'application de la St. V. On remarque que si les effets contralatéraux sont identiques, les effets ipsilatéraux sont différents (meilleure efficacité de la St. V G). Chaque point des courbes représente la moyenne de 320 réflexes limités de confiance à 0.05.

La durée de l'intervalle inter-essais au hasard entre 8 et 15 secondes. Les réflexes H contrôles et les réflexes H conditionnés sont provoqués simultanément dans les deux jambes. Leur présentation suit un ordre pseudo-aléatoire.

Les expériences complémentaires ont été réalisées sur des sujets normaux en plaçant les électrodes de la St. V sur la joue. Les sujets soumis aux mêmes séries expérimentales de stimulation cutanée est identique en durée et en intensité à la St. V. Cette situation contrôlée assure une précaution d'ordre méthodologique. Elle a pour but essentiel parallèlement aux réflexes réalisées sur des sujets labyrinthiques de discuter la spécificité de la St. V et de l'importance des influences aspécifiques de la stimulation cutanée liées à la stimulation électrique de la région otique.

Par ailleurs nous avons employé des tests cliniques classiques pour déterminer la latéralisation motrice des sujets au niveau des membres supérieurs et des membres inférieurs. L'épreuve de la marche aveugle a été utilisée comme test de la dominance vestibulaire après avoir repéré une cible placée à 4 mètres devant eux, les sujets ont pour consigne de marcher vers la cible les yeux bandés. Nous apprécions les déviations à droite ou à gauche dans la trajectoire suivie.

Le traitement des données a consisté en opérations de calcul de la moyenne et de l'écart type des valeurs contrôles. L'amplitude des réflexes H conditionnés a été exprimée en pourcentage de la référence ou en notes réduites Z (c'est-à-dire en écarts-types de la distribution des valeurs contrôles). Les calculs sont effectués par sujet, en fonction du côté de la recep-



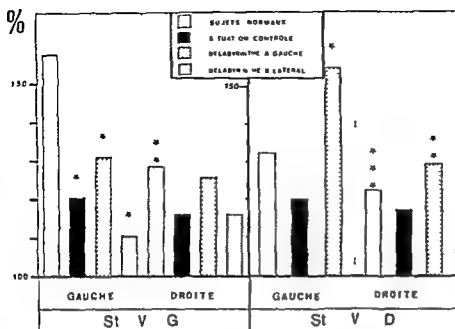


Fig. 3 Diagramme des facilitations des réponses réflexes H conditionnées. Chez les sujets normaux ( $n=20$ ) chez le sujet délabrynthé bilatéral et chez le sujet délabrynthé à gauche les réponses réflexes H sont conditionnées par la stimulation vestibulaire. En situation contrôle, les réflexes sont conditionnés par une stimulation électrique de la peau. Nous comparons la facilitation des réflexes obtenue 100 ms après le début de la stimulation (cutanée ou vestibulaire). L'amplitude des réflexes est exprimée en pourcentage de la référence (réflexes non conditionnés). On distingue 4 effets qui correspondent

aux 4 cases de la figure. 1 effet ipsilatéral et l'effet contralatéral de la stimulation vestibulaire appliquée à gauche (St V G) 1 effet ipsilatéral et l'effet contralatéral de la stimulation vestibulaire appliquée à droite (St V D) \* indique une différence significative ( $P<0.05$ ) dans chaque cas, par rapport au sujet normal. \*\* indique une différence significative ( $P<0.05$ ) entre l'effet ipsilatéral et l'effet contralatéral de la St V. \*\*\* indique une différence significative ( $P<0.05$ ) entre l'effet ipsilatéral de la St V et l'effet ipsilatéral de la St V D.

électromyographique, du côté de la St V et du délai interchocks  $\Delta t$ . Le programme de traitement des données reprend ces diverses opérations pour l'ensemble des sujets.

## RESULTATS

### 1) Modulation de l'amplitude des réflexes H conditionnés

#### a) Chez les sujets normaux

Le déroulement des variations moyennes de l'amplitude des réflexes H conditionnés par la St V est décrit par la figure 2, il diffère selon que la St V est appliquée à gauche ou à droite.

— en St V G (Fig 2A) un effet facilitateur ipsilatéral se manifeste dès 40 ms et culmine à 100 ms où il représente environ 140 % de

la référence (écart de 15 % écart latéralement, la facilitation à 100 ms est significativement

— en St V D (Fig

latérale représente 130

ms (écart de 100

se développe une

à 50 ms, suivie

identique à la

Quel que soit

V, la facilitation

mais elle reste

d'excitabilité.

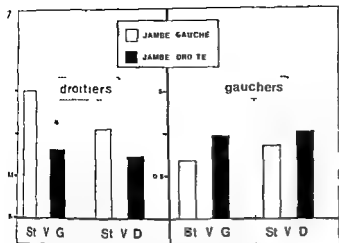
Les réflexes

facilitation

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de la St V

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\* Lateralisation motrice ■ facilitation vestibulaire. Comparaison de la facilitation moyenne des réflexes conditionnés par la St V au délai 100 ms chez les gauchers ( $n=4$ ) ■ chez des droitiers ( $n=12$ ). Les résultats sont exprimés en écarts types de la distribution des valeurs contrôles (notes Z). La facilitation des re-

flexes est plus importante dans la jambe non préférentielle quel que soit le côté d'application de la St V (à gauche St V G ou à droite St V D). \* indique une différence significative ( $P < 0.05$ ) entre l'amplitude des réflexes recueillis sur la jambe gauche et l'amplitude des réflexes recueillis sur la jambe droite.

une inhibition précoce par la St V appli-

■ ■ ■ côté droit

#### Situation contrôle

Les réflexes H conditionnés par une stimulation électrique cutanée de la région des joues (cf figures) sont facilités (Fig 3). La facilitation observée est significativement moins importante que celle provoquée par la St V et ne dépend pas du côté de la stimulation cutanée (gauche ou joue droite). Elle est maximale au délai 100 ms ou elle représente 120% de la valeur de référence. En ce qui concerne les réflexes à gauche et 115% pour les réflexes à droite (différence non significative).

En situation contrôle nous n'observons jamais d'effets précoces facilitateurs ou inhibiteurs.

#### Chez les sujets de labyrinthes

Deux sujets ont été examinés. L'un avait subi une neurectomie vestibulaire chirurgicale thérapeutique à gauche le second est classé cliniquement d'élabyrinthisme bilatéral par barotraumatismes (destruction des organes sensoriels). Les résultats sont reportés dans la figure 3.

La facilitation des réflexes H chez le sujet

de labyrinthe bilatéral est identique à celle obtenue en situation contrôle.

La comparaison des facilitations chez le de labyrinthe à gauche et chez les sujets normaux montre des différences importantes. Chez le de labyrinthe à gauche, la St V appliquée à gauche (côté lésé) est moins efficace et produit des facilitations ipsilatérale et contralatérale modérées et symétriques. Appliquée à droite (côté sain) elle se révèle plus efficace et produit des effets ipsilatéraux ■ contralatéraux asymétriques. La facilitation des réflexes H à gauche est significativement plus grande que chez les sujets normaux. On peut parler d'hyperexcitabilité vestibulaire pour le côté intact et d'hypoexcitabilité pour le côté lésé.

En résumé. La facilitation maximale des réflexes, obtenue au délai 100 ms est asymétrique. Elle est fonction du côté d'application de la St V et est plus importante en moyenne à ■ suite d'une stimulation électrique du labyrinthe gauche. Nous faisons l'hypothèse ■ une prévalence vestibulaire gauche. Par ailleurs quel que soit le côté ■ application de la St V, la facilitation est en moyenne plus grande ■ réflexes recueillis dans la jambe gauche. Cet effet se manifeste également en ■

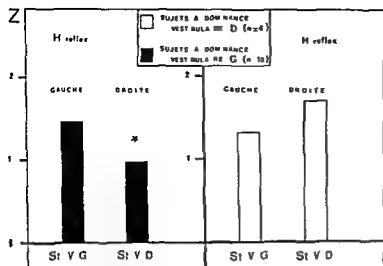


Fig 5 Prévalence vestibulaire et facilitation vestibulo-spinale. Comparaison de la facilitation moyenne des réflexes H conditionnés par la stimulation vestibulaire au délai 100 ms chez les sujets à dominance vestibulaire droite (en noir) et chez les sujets à dominance gauche (en blanc). Les résultats sont exprimés en écarts types de la

distribution des valeurs contrôles (notés Z). Seuls les effets ipsilatéraux sont représentés. On observe que la facilitation des réflexes est toujours plus importante pour la stimulation électrique du labyrinthe dominant. \* indique une différence significative ( $P < 0.05$ ), entre les facilitations de la St V G et la St V D.

trôle (cf Fig 3). On peut penser à une manifestation de la latéralisation des sujets compte tenu que la majorité d'entre eux ont la même dominance motrice droite. La participation de deux facteurs (prévalence vestibulaire et latéralisation motrice) dans la définition de l'effet vestibulo-spinal asymétrique consécutif à une stimulation vestibulaire unilatérale, a été étudiée en regroupant les sujets normaux en fonction de leur latéralisation motrice, puis en fonction de leur dominance vestibulaire observée au plan clinique.

## 2) Latéralisation motrice et facilitation vestibulo-spinale

À l'issue des tests cliniques de latéralisation (cf techniques), 4 gauchers et 12 droitiers ont été retenus. Quatre sujets dont la dominance motrice n'était pas nette ont été éliminés.

On constate l'existence d'une relation entre l'effet facilitateur de la St V sur les réflexes H et la latéralisation: la facilitation la plus importante est enregistrée sur la jambe non préférentielle (Fig 4). La différence entre la facilitation des réflexes H à gauche et à droite est significative ( $P < 0.05$ ).

droitiers, une tendance identique est notée chez les gauchers. Ces résultats sont en accord avec les données classiques qui considèrent que droitiers et gauchers n'ont pas une organisation motrice en miroir.

La plus grande reactivité réflexe de la jambe non préférentielle, quel que soit le côté d'application de la St V, nous amène à considérer le facteur latéralisation comme facteur indépendant, responsable pour une part de l'asymétrie des effets vestibulo-spinaux.

## 3) Prévalence vestibulaire et facilitation vestibulo-spinale

Les sujets sont regroupés selon leur dominance vestibulaire testée par l'épreuve de la main aveugle (cf techniques). En accord avec la clinique nous avons interprété une déviation de la trajectoire vers la droite comme le signe d'une dominance vestibulaire gauche et vice versa. Quatre sujets à dominance droite et 10 sujets à dominance gauche ont été retenus.

Une corrélation étroite est établie entre l'un des paramètres des fonctions vestibulaires observées au plan clinique et la plus ou moins grande efficacité de la St V selon qu'elle est appliquée sur

côté fonctionnellement dominant ou au côté fonctionnellement dominé : la stimulation électrique du labyrinthe dominant provoque une facilitation des réflexes H ipsilatéraux plus importante que celle évoquée par la stimulation du labyrinthe dominé (Fig 5)

Chez les sujets classés à dominance vestibulaire gauche, la facilitation obtenue, par stimulation du labyrinthe gauche, sur la jambe gauche, est significativement plus grande que la facilitation obtenue par stimulation du labyrinthe droit sur la jambe droite

Chez les sujets classés à dominance vestibulaire droite on observe, en fonction de la dominance, une différence des facilitations ipsilatérales qui va dans le même sens sans toutefois être significative

Cette sensibilité différentielle des structures labyrinthiques à la stimulation électrique continue à délimiter, indépendamment de la lateralisation motrice, des effets spinaux asymétriques

## DISCUSSION

### 1) *Effet spinal de la stimulation électrique et structures nerveuses centrales impliquées*

Le recours à la pathologie humaine du labyrinthe nous permet de préciser le point d'impact de la St V. Une lésion du système vestibulaire périphérique (récepteurs sensoriels) rend dans nos conditions la St V inefficace (cas du sujet délabyrinthe bilatéral). Une section complète de la branche vestibulaire du VIII (cas du sujet de labyrinthe à gauche) réduit considérablement l'efficacité de la St V appliquée au côté lésé. Nous avons récemment entrepris des neurotomes vestibulaires unilatéraux chez le Singe (*Papio papio*). Les résultats préliminaires obtenus en employant les techniques utilisées chez l'Homme et les mêmes paramètres de stimulation, indiquent que la St V appliquée au côté lésé n'influence pas l'excitabilité réflexe spinale par contre les réflexes H sont normalement facilités lorsque la St V est appliquée au côté sain. Pour des intensités élevées (de l'ordre de 6

à 8 mA), la stimulation du côté lésé retrouve son efficacité et provoque une modulation de l'excitabilité spinale

Ces résultats nous permettent de conclure au point d'impact périphérique et sensoriel de la St V pour les intensités de stimulation employées chez l'Homme (2,5 mA), et à une atteinte moins sélective du système vestibulaire pour des intensités élevées (excitation directe du nerf vestibulaire). L'hyperexcitabilité observée par stimulation du côté sain chez le sujet délabyrinthe unilatéral paraît liée au développement d'un mécanisme central de compensation. Selon Pfaltz & Piffko (1972), un mécanisme possible de la compensation serait basé sur la modulation de la réactivité de l'organe sensoriel intact au moyen de fibres efferentes. Cette hyperexcitabilité existe chez le Singe dans les 3 ou 4 jours qui suivent l'intervention chirurgicale.

L'effet spinal de la St V consiste essentiellement en un relèvement de l'amplitude des réflexes H. C'est une facilitation brève mais importante de la réactivité réflexe spinale, principalement ipsilatérale, qui culmine à 100 ms ou elle représente au maximum 160% de la référence. Dans nos conditions expérimentales, cela correspond à un recrutement d'environ 15% de la frange motoneuronale activée subliminairement par la volée afférente réflexogène. La comparaison des résultats obtenus en situation contrôle, chez les sujets normaux et dans la pathologie, nous renseigne sur la nature de l'effet spinal de la St V. La facilitation des réflexes H, 100 à 150 ms après le début de la St V, résulterait de la combinaison de deux influences mises en jeu par la stimulation électrique par voie externe : l'une, spécifique, correspondrait à l'activation des récepteurs sensoriels labyrinthiques, l'autre, aspécifique, serait liée à la stimulation électrique de la peau.

Nous interprétons la facilitation des réflexes en situation contrôle et chez le sujet labyrinthe bilatéral comme le signe d'un reticulaire aspécifique à point de départ. Cette composante reticulaire des réflexes a été souvent

par Paillard (1955) qui étudiait la modulation du réflexe H conditionné par un son, il concluait à une action sur les motoneurones spinaux par les mêmes circuits que ceux qui organisent la réaction de sursaut.

Les voies nerveuses empruntées par les influences spécifiques d'origine vestibulaire ne sont pas précisées par cette étude. Le délai nécessaire à l'activation des structures labyrinthiques par la St V n'est pas connu, nous ne pouvons qu'émettre des hypothèses sur le problème de l'organisation des liaisons vestibulo-spinales chez l'Homme.

Le faisceau vestibulo spinal latéral, originaire du noyau de Deiters ipsilatéral atteint les régions lombaires; il peut constituer une voie d'expression des influences vestibulaires spécifiques. L'existence de projections vestibulo-réticulaires suggère que les voies suivies pourraient être vestibulo-réticulo-spinales. Ce décours réticulaire expliquerait pour une part l'effet contralatéral de la St V.

L'intervention du cervelet est à considérer des fibres vestibulaires primaires projettent directement sur le lobe flocculo-nodulaire. Les travaux de Brodal (1960) soulignent la richesse des connexions entre le système vestibulaire et le cervelet et montrent, au moins chez le Chat, l'existence d'un système d'afférences bien organisé. Schématiquement, on distingue deux ensembles fonctionnels sur chaque noyau vestibulaire: l'un recevant des afférences vestibulaires primaires, l'autre ou convergent des influences en provenance du noyau fastigial et du cortex cérébelleux (pour le noyau de Deiters, cette dernière région est en relation avec les noyaux moteurs des membres postérieurs). Les connexions sont principalement ipsilatérales. À partir du noyau fastigial, elles devraient être facilitatrices, à partir de l'archécervelle inhibitrices. On comprendrait alors que des effets opposés puissent s'exprimer aux délais précoces 30 et 50 ms et la facilitation des réflexes H, 100 à 150 ms après le début de la St V représenterait le rebond classique de facilitation consécutif à une activation cérébelleuse.

## 2) Rôle de la latéralisation motrice

Les travaux de réflexologie conduits habituellement que la réactivité réflexe au niveau des membres postérieurs est identique. L'étude de courbes de recrutement du réflexe H et de courbes d'excitabilité effectuées à droite et à gauche ne montre pas de différence significative. Par contre, les effets spinaux de la St V dépendent en partie de la latéralisation: chez les sujets quel que soit le côté d'application de la St V, la facilitation du réflexe H est plus importante en moyenne pour la jambe préférentielle.

Nous pensons que l'asymétrie de la réaction spinale exprime la dominance hémisphérique motrice. Chez l'Homme en effet, la qualité des performances motrices que réalisent chacun des membres est inégale, en particulier, les membres postérieurs ont un rôle fonctionnel déterminé dans l'ensemble des habitudes motrices et des gestes automatisés du comportement locomoteur. Les différences fonctionnelles observées font supposer que les contrôles supraspinaux qui s'appliquent à des noyaux moteurs spécifiques n'ont pas la même efficacité. Ces contrôles seraient plus importants sur l'hémicorps dominant; si l'on est droitier, ils détermineraient la dominance des motoneurones aux influences périphériques et centrales.

Nous avons récemment repris l'étude de l'asymétrie de la réactivité réflexe postérieurs (Bonnet & Lacour, 1970) portant sur les effets d'un son binaural sur les réflexes de Hoffmann, de composition de droitiers et de gauchers sélectionnés. Une facilitation H est observée, interprétée comme une facilitation réticulaire. On constate une asymétrie: la facilitation est significativement plus importante pour la jambe non préférentielle et la facilitation est plus faible.

Les résultats qu'apparaissent sont interprétables: l'activité des structures supraspinales agit sur la variabilité du système composé de

est d'autant plus faible qu'il y a indépendance entre les éléments qui le composent. L'introduction d'une corrélation, même faible, entre plusieurs de ces éléments (par exemple l'action d'une cause commune), accroît la variabilité de la réponse globale du système. On peut concevoir que toute manifestation de contrôle de l'activité du noyau moteur par les structures centrales, qui tend à faire corréler l'activité des motoneurones, a pour résultat d'augmenter la variabilité de la réponse globale. En termes plus physiologiques, on pourrait dire que l'importance de la variabilité des réponses réflexes sur la jambe préférentielle, traduirait l'existence de facteurs corrélants du niveau d'excitabilité des motoneurones, c'est à dire l'existence de contrôles centraux plus importants et efficaces que sur la jambe non préférentielle.

On comprendrait alors l'existence d'une plus grande réactivité à la St V des réflexes H de la jambe non préférentielle, leur plus grande sensibilité aux influences vestibulaires. Le supranatomo-fonctionnel de ce contrôle cortical différentiel sur des structures spinales symétriserait celui de l'asymétrie pyramidale, génétiquement déterminée pour une part, et renforcée par les processus d'apprentissage.

#### 4) La prévalence vestibulaire

Chez la majorité des sujets le labyrinthe gauche est plus réactif à la stimulation électrique. La facilitation du réflexe H est significativement supérieure en moyenne lorsque l'on stimule le labyrinthe gauche. Ce résultat ne paraît pas dépendre de la latéralisation motrice des sujets. Si l'on regroupe les sujets selon leur dominance vestibulaire testée par l'épreuve de la marche aveugle, on constate (Fig. 5) que la facilitation des réflexes ipsilatéraux est plus importante lors de la stimulation du labyrinthe dominant. La forte proportion, dans notre échantillon, de sujets à dominance vestibulaire gauche explique l'observation globale d'une prévalence du système vestibulaire gauche.

Dans leur étude des déviations latérales de l'axe corporel provoquées par une stimulation galvanique du labyrinthe, Corns et

(1972) concluent également à une prédominance du système vestibulaire gauche. Ils notent un nombre significatif d'inversions de la direction des oscillations latérales du corps lorsqu'ils inversent la polarité de la stimulation du labyrinthe gauche: la polarisation anodique ou cathodique du labyrinthe droit n'affecte pas le sens des déviations. Les auteurs comparent ensuite la direction de la rotation qui provoque une réponse nystagmique de plus haute fréquence à la direction de la déviation de l'axe corporel provoquée par leur stimulation galvanique; ils montrent une corrélation positive entre la rotation horaire (à droite) et la stimulation cathodique du labyrinthe gauche (stimulation qui évoque une déviation vers la droite). Ils n'observent pas de corrélation entre le sens de la déviation et la latéralisation motrice des sujets. D'autres travaux (Milojevic & Watson, 1965), relatifs à la réponse nystagmique au test calorique, indiquent une « prépondérance directionnelle » pour la stimulation du labyrinthe gauche. Cette réponse nystagmique plus importante à la stimulation calorique (44°) de l'oreille gauche se manifeste chez des droitiers et chez des gauchers. De la même façon, notre étude n'établit pas de relation nette entre la prévalence vestibulaire et la latéralisation, mais une tendance se manifeste: on rencontre plus fréquemment l'association (dominance motrice droite — prévalence vestibulaire gauche).

Deux questions se posent au sujet de la prévalence vestibulaire:

quels mécanismes peuvent rendre compte de la sensibilité différentielle des récepteurs labyrinthiques à la stimulation électrique?

Quelle serait la signification fonctionnelle d'une prévalence vestibulaire?

La réponse à la première question peut s'appuyer sur les données de Gerhardt (1967). Au plan anatomique, l'existence d'un faisceau de fibres efferentes a été démontrée au niveau des récepteurs labyrinthiques: au plan fonctionnel, ce contingent efferent serait capable de régler la sensibilité des récepteurs. Un tel contrôle efferent se retrouve en effet dans la majorité des

bulaire, il serait essentiellement réalisé par l'intermédiaire de fibres non croisées atteignant les récepteurs et les noyaux vestibulaires. Son origine corticale ou sous-corticale est controversée, son action inhibitrice probable. Nous faisons l'hypothèse qu'une intervention différentielle de ce contrôle sur les structures vestibulaires symétriques est à l'origine de la différence de réactivité constatée. Par ailleurs, on peut envisager que la réponse sensorielle labyrinthique est identique de chaque côté mais qu'elle s'exprime à travers des structures latéralisées. La richesse des connexions vestibulo-cérébelleuses suggère qu'il existe peut-être une relation entre asymétrie vestibulo-spinale et asymétrie cérébelleuse. L'emploi de tests cliniques purement cérébelleux serait de ce point de vue à envisager.

L'action différentielle d'un contrôle cortical (ou sous-cortical) sur des structures réceptrices sensorielles symétriques est une hypothèse plus séduisante. Si elle se trouvait confirmée, elle entrerait dans le cadre des données relatives à la sensibilité différentielle des récepteurs pairs. Un certain nombre d'asymétries auditives ont été

en évidence chez l'homme, en particulier l'avantage perceptif de l'oreille gauche pour les sons non verbaux (Botte et Chocholle, 1972). De la même façon, on constate l'existence d'une dominance oculaire. Il semble que dans chaque couple qui constituent les systèmes sensoriels symétriques, se manifeste une asymétrie fonctionnelle. La prévalence observée pour le système vestibulaire pourrait relever d'une organisation analogue.

SUMMARY

The organization of postural reflex reactions following labyrinthine excitation was taken up in man by studying interactions between descending vestibular influences and spinal activities involved in segmental proprioceptive reflexes. 20 normal and 2 abnormal subjects were examined: they were submitted to a monaural galvanic vestibular stimulation checked by the H reflex amplitude and to both motor and vestibular testing. The results indicated an asymmetrical increase in the ipsi- and contralateral H reflex variations depending on the essentially homolateral vestibulo-spinal connections: on a more important reflex reactivity in the non preferred leg and

on a unilateral vestibular prevalence. The motor dominance and asymmetrical reflex reactivity in the H reflex was discussed referring to a differential central control over symmetrical effector and receptor structures.

ZUSAMMENFASSUNG

Die Autoren untersuchen die Beeinflussung der Erregbarkeit spinaler Reflexschaltkreise (monocortikaler Reflex nach Hoffmann) durch monaurale vestibuläre Vestibularreize. Diese Reizung provoziert eine Erhöhung der Amplitude des H Reflexes. Auf der linken Seite bewirkt sie im Mittel eine Bahn um 13% größeren Ausmasses. Die Hypothese einer vestibulären Dominanz wird diskutiert. Dieses Ergebnis hängt eng mit der Richtungsabweichung von Versuchspersonen im Gehversuch mit verbundenen Augen zusammen. Die Analyse der Ergebnisse weist ausserdem auf unterschiedliche Reaktivität in beiden Beinen hin. Die Bahn des H Reflexes ist im nicht dominanten Bein immer bedeutender. Die Begriffe der vestibulären Dominanz und der Asymmetrie der Reflexreaktivität in den verschiedenen Extremitäten werden im Rahmen einer differenziellen Kontrolle auf die zentralen Ursachen und symmetrische motorische und sensorische Strukturen hin diskutiert.

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## SUBTOTAL EVULSION OF THE FACIAL NERVE IN HEMIFACIAL SPASM

J Sade

*From the Meir Hospital, Nfar Saba, Israel*

(Received February 19, 1974)

**Abstract** The various surgical procedures performed for hemifacial spasm whereby part of the facial nerve is sectioned indicate (1) An intermingled intrafacial arrangement of the various nerve filaments. This arrangement differs from the classical concept that there is a specific regional representation of the peripheral branches and of their tracts in the main trunk. The intermingling of all peripheral filaments in the main trunk explains why even a small portion of the main trunk may represent functionally most of the face. (2) The presence of a wide peripheral anastomosing network connecting the peripheral branches in such a manner that one branch may functionally represent the other branches if they are severed.

On the basis of these anatomical observations and because of the frequent recurrence of facial spasm after most prescribed surgical procedures, evulsion of most peripheral twigs is recommended in certain cases. The mandibular branch and one or two superior branches are left. This is considered a compromise surgical procedure, resulting in only slight facial weakness as well as possible recurrence of mild and relatively sparse spasmodic movements.

Further theoretical conclusions from the intrafacial fascicle arrangements constitute *a priori* limitation of surgical results from facial nerve anastomosis and grafting in traumatic cases.

Hemifacial spasm is a disease characterized by involuntary one-sided tonic- and clonic facial movements. In the early stages the spasm occurs at infrequent intervals, mostly around the orbicularis oculi. However, in time most of the facial muscles often become involved (Fig. 1) the spasm recurs at more frequent periods—up to many times a day—and has the appearance of a facial "mass movement", thus constituting a formidable nuisance. The disease is usually seen in adulthood and should be differentiated from nervous tics, post-traumatic tics or spasms sec-

ondary to a central nervous lesion. True hemifacial spasm can also appear during sleep (Greenwood, 1946, Potter, 1972) and cannot be suppressed voluntarily, whereas a nervous tic does not appear during sleep, can be controlled to some degree, and is usually not confined to the face. The nerve. Traumatic tics (Fowler, 1939) have by definition a traumatic history, while those secondary to central nervous lesions are of a tonic in character and are associated with other central nervous symptoms. Hemifacial spasm has been well reviewed by Ehni & Wolman (1945) and O'Donnell (1953).

The number of treatments suggested for this disease are many, as is the case in diseases for which there is no really satisfactory treatment. Tranquilizers, muscle relaxants and physiotherapy have not been found helpful (Greenwood, 1945, McCabe 1970). Dividing the facial nerve and anastomosing it to the hypoglossal or spinal accessory was found to stop the spasm (Ehni & Wolman, 1945) but the loss of satisfactory facial control by the patient makes the method unacceptable. Rhizotomy was attempted and abandoned (Ehni & Wolman, 1945).

Injection of denaturing compounds such as phenol or alcohol (Greenwood, 1946, Wakasugi, 1972) into the peripheral branches of the nerve or into its main trunk is rather popular because of the technical simplicity of the method and initial effectiveness, however the beneficial effect is only temporary and recurrence is the rule (McCabe, 1970, Wakasugi, 1972). Subsequent injections are often less successful possibly due



Fig. 1 Hemifacial spasm. Patient showing involuntary contraction of the right side of her face—note this movement resembles a "mass movement".

a perineural fibrosing reaction which also makes subsequent surgery rather difficult (Sade

In various nerve resection surgical methods were directed both at the main stem and the peripheral branches. Total division or sectioning of the main stem was practised by Rathbone (1956) who reported his patients were free from spasms as long as the face was analysed but that the spasm recurred once the nerve regenerated. Opinions vary as to which method is the more desirable—some prefer the resection rather than the paralysis and others vice versa. Interestingly relief from spasm was also obtained after facial nerve decompression. More physiological and interesting measures were introduced by German (1942) who sectioned surgically parts of the various peripheral branches (Fig. 2)—this technique was later followed by Greenwood (1946) Miehke (1959) and

Diamant et al (1967). The initial results were satisfactory but the rate of recurrence after necessitated further operations which were not always easy to perform because of scarring.

Scoville (1955) advocated a variation of this procedure—thinning out the main facial stem (Fig. 2) he cuts down to at least 2/3 of its depth to avoid a high recurrence rate. Already in 1946 Greenwood advocated selective resection of some of the peripheral branches—this procedure was later taken up by Fisch (1977) though Boyd (1972) maintained that the branches should not merely be cut but actually evulsed.

McCabe (1970) pointed out that all the above mentioned methods invariably end in recurrence of the spasms and advocated therefore evulsion of the nerve—but for its mandibular and cervical branches. Such an operation results actually to an upper facial paralysis (eye and upper lip) and while this is a disadvantage as the spasm is concerned, it is probably the cost might seem to be justified.

We have treated patients with spasms by phenol injections as well as by partial resections of the facial nerve (by partial longitudinal resection and some branch amputation). Initial amelioration of the condition was followed usually by a longer lasting relief without having to resort to a more extensive procedure. Such a method is reported here as well as what can be learned from the various facial nerve surgical procedures with regard to the intimate axonal intrafacial neuroanatomical arrangements.

## SURGICAL METHODS AND PROCEDURES

### *Subtotal facial nerve evulsion—rationale and procedure*

We are ignorant of the reason for the initial therapeutic benefit provided by the various facial nerve resection and dilution techniques (chemical or surgical)—as well as to why the recurrence of spasms. However one observation is that the spasmodic recurrences are often (though not

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McCabe (1970) pointed out that all the above-mentioned methods invariably end in recurrence of the spasms, and advocated therefore total evulsion of the nerve—but for its mandibular and cervical branches. Such an operation amounts actually to an upper facial paralysis (forehead, eye and upper lip), and while its result as far as the spasm is concerned is probably very good, the cost might seem to be rather high.

We have treated patients in our Department by phenol injections as well as various partial resections of the facial nerve branches (both partial longitudinal resection and selective whole branch amputation). Initial amelioration of the condition was followed usually by a recurrence. Therefore, I looked for a method of providing longer lasting relief without having to resort to 'above lower lip' facial paralysis. Such a method is reported here as well as what can be learned from the various facial nerve surgical procedures with regard to the intimate axonal intrafacial neuroanatomical arrangements.

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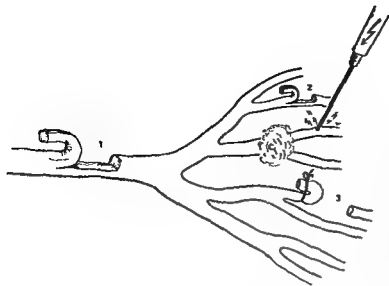


Fig 2 Various surgical treatments formed for hemifacial spasm (1) Thinning the main stem (Scoville) (2) Longitudinal splitting of the peripheral branches (man, Greenwood Michlke Di et al) (3) Selective cutting of peripheral branches (Greenwood Fisch) C, cotton with cocaine to block retrograde electric impulses when the extent of stimulus is tested

always) more or less in proportion to the amount of nerve tissue sacrificed initially

Clearly, the recurrent spasm after some peripheral branches are sectioned (Fig 2) must be through an efficient peripheral anastomosing network, which we do not usually see when exposing the facial nerve in the parotid (Fig 3)

The notion that recurrence is due to regrowth of the nerves can be dismissed because we bend the stumps backwards (Fig 2) Furthermore the fact that no selective regional facial paralysis occurs after removal of several peripheral facial nerve branches would indicate that the remaining branches take over for those which have been sectioned, which would only be possible through a rather extensive peripheral anastomosing network Further evidence as to the existence of such a network (at least in cases of facial spasm) can be found on electrical stimulation of selective peripheral branches In this case, we have observed mass movement of all, or most of, the face, and not only muscle contraction of the narrow sections to which the particular branch is leading The possibility that such stimulation reached the other branches *retroactively* was eliminated by cocainization of the "hind" part of the nerve, thereby blocking retrograde impulses (Fig 2) We obtained the same result, indeed, also after the connection of that particular branch was severed from the main stem

Our surgical results and the rate of recurrence should therefore related not only to the number of twigs resected but also to the remaining anastomosing peripheral network Better results are achieved therefore if we *evulse* the nerve branches, as advocated by Boyle (1972)

The operation is done through a parotidectomy skin incision and a rather full exposure of the facial nerve—its main trunk and the peripheral nerve fibres (Fig 3A and B) No parotid gland tissue is sacrificed, however The operation consists of The pulling out, extirpation or evulsion of the individual facial branches, instead of resecting them, rolling them on a hemostat "a la spaghetti" as much as possible before pulling them out—and thereby removing a nerve which is several centimetres long, in the hope that many of the peripheral anastomosing branches and their connections will be pulled out or severed In this fashion, all the peripheral facial branches are eradicated except (1) The cervical branch—which usually plays an unimportant role in the spasmodic syndrome, (2) the mandibular branch which usually also plays only a relatively minor part in the spasmodic syndrome and which apparently has a poor (or no) anastomosing relationship with the other nerve fibres—as evidenced also by its weakness when it is inadvertently injured while performing a submandibular gland extirpation, (3)



**Fig 3A** Exposure of facial nerve in the patient. Main trunk at dividing point. 'X' small arrows show branches to be evulsed. Large arrows indicate branches left intact.



**Fig 3B** Same patient as 3A after evulsion of branches. Silk sutures are seen as markers at points where the nerves were cut.

one or two of the uppermost twigs (Figs 3A and B). It is this latter sole twig (or two) which will take over later adequate facial closure of the eye, some movement of the upper lip and preservation of facial tone. However, it will also be responsible for the possible recurrence of some weak spasmodic episodes—constituting the compromise—a fair price for the avoidance of upper facial paralysis. Whether to leave one or two twigs depends on their thickness—some information might also be provided through the nerve stimulator but essentially the decision will depend on the experience and judgement of the surgeon.

The facial nerve stimulator is not absolutely necessary for the performance of the operation though it is a comfort to have such a tool which helps inform the surgeon at each step while progressively cutting and evulsing the peripheral branches that what has been left behind is still adequate for moving the muscles of all or most

of the face—leaving even a weak response to suffice for adequate movement later on.

#### *Postoperative period and aftermath*

Many of the facial nerve dilution operations as well as the phenol alcohol techniques lead to an initial stage of facial weakness if not actual facial paralysis. In all these procedures the facial nerve usually returns to normal within days or weeks<sup>1</sup> and the spasm disappears—only to reappear usually before a year is over.

In the operation described above, subtotal facial nerve evulsion, facial weakness may also set in immediately postoperatively. It disappears entirely or to a great extent within weeks or a few months. The facial spasm however disappears immediately. When the period arrives at which

<sup>1</sup> In one of our patients such facial paralysis lasted hours while in another it lasted over 2 months. In cases, the treatment was injection of p. main trunk region (C. G. G. 1973).

the facial spasm usually reappears, i.e. after about 6-12 months, slight spontaneous movements may occur now and then. These symptoms are, however, so slight that they are hardly troublesome to the patient—by no means anything like the original condition. Altogether, subtotal evulsion of the facial nerve is therefore an operation which in the long run, may deprive the patient of a small part of his facial integrity and of the greater part of his spasm—a compromise between operations giving a more severe recurrence with no facial weakness, and operations giving no recurrence at all but significant facial weakness.

The results of treatment in hemifacial spasm should be evaluated post-operatively after at least 2 years—at that time all that could recur will already have set in. I have observed 5 patients (2 men, 3 women) treated by subtotal evulsion of the facial nerve for a period of over 2 years. Their average age is 54 years and the average post-operative time 31 months. Two of them had slight facial weakness post-operatively for some weeks, two had moderate weakness for 2 months and the fifth had significant facial weakness for 5 months. In all cases facial movements have returned, though a very slight facial asymmetry remains—which however is mostly observed only by the initiate, and does not disturb the patient. The facial spasm disappeared immediately in all 5 patients—though after 9-12 months slight spontaneous movements returned in three. These appear rather infrequently and leave these patients undistressed. The fifth patient—who had the most severe postoperative facial weakness—has had no recurrence of spontaneous movement. As all these patients are now over 2 and close to 3 years postoperative, the chances are that these results are stable.

## DISCUSSION

### *The nature of hemifacial spasm*

Though fatigue and tension may aggravate the spasm, this entity is not considered to be of psychosomatic origin because of its strict and neurological localisation, lack of response to

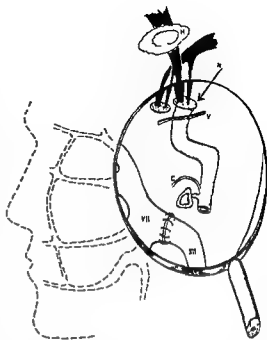


Fig 4 Schematic drawing to show why the origin of spontaneous electric charges in hemifacial spasm is most likely nuclear VII, peripheral part of facial nerve XII hypoglossus anastomosed to peripheral part of facial nerve, C, chorda tympani, V, blood vessel near brain stem (Jannetta) N, facial nucleus H hemorrhage (Ehm & Wolfman)

tranquilizers or psychotherapy and the presence of contractions during sleep (I have even seen the face contract under general anaesthesia!).

The disappearance of the contraction after facial nerve resection with or without hypoglossus anastomosis precludes the origin of the spasm as occurring at a region peripheral to the stylo mastoid region (Fig 4). Diamant et al.'s (1967) observation of concomitant stapedial contraction as well as hyper-secretion from the submandibular glands with facial spasm places the lesion above the second facial genu. How high above the genu do the spontaneous electrical discharges originate? (Fig 4) Do they originate centrally as in epilepsy or more peripherally, as in spontaneous vertiginous attacks in Meniere, or as in painful discharges from trigeminal neuralgia? The occasional beneficial effect of Tegretol or antiparkinsonian drugs—which I have seen in some but not all patients, suggests a nuclear origin of the spontaneous

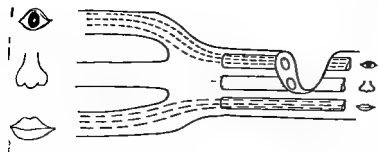


Fig 5 Diagram showing the specific regional representation theory whereby each peripheral branch represents a specific facial region and that such a representation continues in the main facial stem

Échange (Bloom, 1963) At the same time, evidence which eliminates the cortex as source of the spasms—thus 2 patients affected with facial spasm suffered a stroke involving the face but not affecting the spasm (Ehni & Woltman, 1945) There is also experimental work supporting the non involvement (Fowler, 1939, Ehni & Woltman, 1945) of the The main evidence as to the trigger zone, elusive as it is, indicates the region above second facial genu up to the facial nucleus the pons as that where the spontaneous electrical activity originates Jannetta's (1970) observation of blood vessels compressing the intracranial part of the facial nerve is in agreement with this belief Thought, however, should also be given to the theory that the 4-S system—a system which controls much of our finer movements—is defective (McCabe, 1970)

Facial nerve anatomy, as seen through surgery for hemifacial spasm

The peripheral branches of the facial nerve are believed traditionally to carry impulses to defi-

nite and specific regions of the face (May, 1970) The same representation is supposed to be present in the main facial trunk throughout its length, (including also the middle ear) We shall term this concept the *specific regional representation theory* (Fig 5)

The operations performed for facial spasm on the main trunk (Scoville, 1955) of the facial nerve consist essentially in dividing an important part of its width together with the fibres which pass through it (Fig 2) This operation would bring about a total *selective* or *regional* paralysis of those parts enervated by the cut fibres, if the specific regional representation theory reflected the intimate anatomy of the facial nerve trunk However, such selective paralysis does not happen

Furthermore, as mentioned previously, those operations which sacrifice several of the peripheral facial nerve branches would also lead to specific regional paralysis if each branch were to represent a specific region—and this does not happen either The observation that no specific regional facial paralysis occurs, either when the

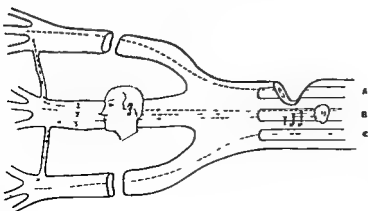


Fig 6 Diagram showing peripheral anastomosis through which pass the result of which representation of the peripheral innervation



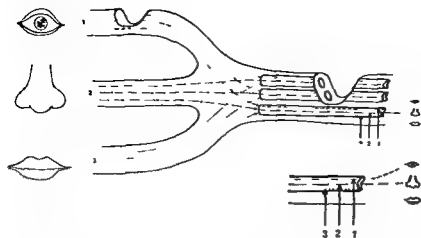


Fig 7 Diagram showing the intermingling and intermixing of various peripheral branches in the main stem which bring about wide representation (fibres from 1 & 2 & 3) by even a small nerve bundle in the main stem

main trunk is partly divided or when some of the peripheral branches are divided, should therefore lead us to reject the specific regional representation theory. What seems to be the case is that each peripheral branch "represents" a large part of the whole face. When some are cut, the remaining one or two suffice to carry movements to the entire face—this can probably come about, as already mentioned, only through a very extensive peripheral anastomosing network (Fig 6). At the same time the fact that only a small section of the main trunk also suffices to enervate the whole face indicates that each small section of the main trunk also has enough representative axons to represent a major part of the face. This could be achieved through an intraneural arrangement of axonal mixing and intermingling, so that the various peripheral regions represented in the main trunk are arranged in several bundles—each of them representing the whole face or its greater part (Fig 7).

These anatomical conclusions derived from the various surgical observations have also received conclusive support from the experimental work of Duel & Fowler (1939) as well as experiments of Harris (1968) and from microanatomical dissections of the facial nerve, by Sunderland & Cossar (1953), and White & Verma (1973).

This subdivided distribution of the various axons into a multiple bundle arrangement—each bundle carrying axons for the whole face—has another interesting aspect. A serious doubt

arises as to whether such a very complicated and sophisticated micro arrangement can be approximated surgically, after being traumatically severed. The "high distribution factor" of each of the fibres in the trunk makes exact matching of single nerve sections highly improbable. The traditional specific regional representation concept allows for a more or less approximation of the various intraneural specific regions" when a traumatic severed main trunk is resutured. However, if we consider this specificity to be widely distributed among various bundles and limited more to the individual axons than to a whole group of them, surgical approximation becomes a rather remote practical possibility. This consideration should have a bearing on the perfection or imperfection of the results of facial nerve suture or grafting in traumatic cases, and explain much of the associated and mass movements observed after such reconstructive surgery—as perfect as it may seem to be technically.

It is also not certain that the amelioration seen after single severed peripheral facial branches are sutured is actually due to this surgical procedure and not to messages arriving through peripheral anastomosis.

#### *Treatment of hemifacial spasm*

This affliction can be slight or severe and the patient's attitude to his ailment varies also from relative nonchalance and acceptance to complete fixation on the contractions and to the attention

which the community pays to this phenomenon spontaneous cure is indeed a rare occurrence (Ehru & Woltman, 1945).

After having observed and treated 21 patients, I do not believe that all cases should be treated in the same way. The mild cases should be treated—at the beginning at least—with Tegretol, while in more advanced cases in elderly patients phenol injection into the facial trunk should be considered. Severe cases appearing in relatively young, active, people should be treated by surgery. When proposing surgery, one should explain to the patient the nature of the disease and the factors involved, i.e., spasms, paralysis and recurrence and the compromise described above, of—the possibility of barely perceptible and slight facial weakness associated with occasional recurrence of mild spasms—as well as the other treatments and alternatives.

### ACKNOWLEDGEMENT

I would like to thank Mr Ben Jahd for his help in making the drawings and Mr Falik for the photography.

### ZUSAMMENFASSUNG

Die verschiedenen chirurgischen Verfahren, die im Falle eines hemifazialen Spasmus ausgeführt werden, wobei ein Teil des fazialen Nervi sezziert wird, zeigen an 1. Eine vermischte intrafaziale Anordnung der verschiedenen Nervenfilamente. Diese Anordnung weicht von der klassischen

funktionell einen grosseren Teil des Gesichtes darstellen kann 2. Die Anwesenheit eines weiteren peripheren anastomotischen Netzwerkes, das die peripheren Zweige in solcher Weise verbindet, dass ein Zweig funktionell die anderen Zweige im Falle einer Abtrennung repräsentiert.

Auf Grund dieser anatomischen Beobachtungen und den häufigen Rezidiven der fazialen Spasmen nach meist vorgeschriebenen chirurgischen Eingriffen wird in manchen Fällen die Ausrottung der meisten peripheren Zweige empfohlen. Der mandibuläre Zweig und ein oder zwei oberer Zweige werden zurückgelassen. Dies wird als chirurgische Kompromissverfahren betrachtet, das eine leichte Fazialschwäche sowie auch mögliche, milde

und verhältnismässig seltene spasmodische Bewegungen ergibt. Weitere theoretische Folgerungen aus der Anordnung der fazialen Filamente bedeutet, a priori eine Begrenzung der chirurgischen Ergebnisse der fazialen Nervenverbindung und Verpflanzung in traumatischen Fällen.

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## CAPILLARY AND SHUNT BLOOD FLOW IN THE NASAL MUCOSA OF THE CAT

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(Received February 15, 1974)

**Abstract:** Radioactive microspheres ( $15 \pm 5 \mu\text{m}$ ) were used to investigate capillary and shunt blood flow in the nasal mucosa of the cat. Capillary flow was about 0.5 ml/g min. An abundant arterio-venous shunting was simultaneously recorded. Shunt flow was  $57 \pm 11\%$  of total blood flow. Sympathetic stimulation resulted in a greater reduction in shunt flow than in capillary flow, indicating that increases in sympathetic nerve activity will result in a redistribution of flow from shunt to exchange vessels.

Morphological studies of the nasal vascular bed in man and cat have demonstrated the presence of arterio-venous anastomoses (AVA) in the nasal mucosa (Dawes & Prichard, 1953, Rossetti, 1954, Cauna, 1970). The AVA were frequently encountered in the deeper sections of the nasal respiratory mucosa and usually near the nasal glands (Fig 1A).

Previous experimental studies on the microcirculation in the nasal mucosa have provided information regarding the nervous control of resistance, capacitance and exchange vessels (cf Änggård, 1974, Malm, 1973). However, no information is available on the quantitative importance or the nervous control of arterio-venous shunt flow in the nasal vascular bed.

Injection of radionuclide-labelled microspheres into the circulation of animals provides a possibility of measuring the proportion of blood flow to any organ or the regional distribution of the blood flow within an organ (cf Rudolph

& Heymann, 1972, Wagner et al., 1969). An important assumption for the technique is that all microspheres are trapped in the small vessels. If AVA are present these will allow an escape of the spheres, which will then appear in the venous effluent in proportion to shunt flow.

In the present investigation this method was used to demonstrate the presence and quantitative importance of shunt flow in the nasal vascular bed of the cat.

### MATERIALS AND METHODS

Experiments were conducted on 17 cats (2.3-4.5 kg) anesthetized with chloralose-urethane (50 mg/kg + 100 mg/kg i.v.). A diagrammatic presentation of the experimental arrangement is shown in Fig 1B. The trachea was cannulated. The pressure in the left femoral artery was measured by a Statham pressure transducer (P23A) and recorded on a Rikadenki multichannel recorder. Rectal temperature was kept constant at  $38^\circ\text{C}$  by heating lamps. The right cervical sympathetic nerve was dissected free from the vagal nerve and transected. Sympathetic stimulation was performed in the cranial direction using a bipolar silver electrode. The stimuli were monophasic square wave pulses (6V, 1 msec) delivered by a Grass model S4 stimulator. Stimulation frequencies of 0.5 and 10 imp/sec were used. For the collection of blood from the nose the right sphenopalatine vein, which drains the

This investigation was supported by grants from Karolinska Institutet, Stockholm, and Svenska Sällskapet för Medicinsk Forskning.

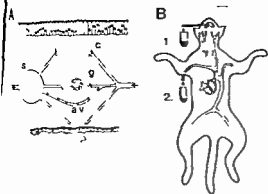


Fig. 1 Schematic drawing of the experimental set up and the vascular arrangement in the nasal mucosa.

rocephalic trunk with certainty, the left atrium was cannulated in later experiments. In 9 cats artificial respiration was given (B Braun Melsungen respirator) and a left sided thoracotomy was performed. A polyethylene catheter (PE 20) was introduced into the left atrium through a small pulmonary vein. Arterial blood samples were taken repeatedly and analysed for pH,  $p\text{CO}_2$  and  $p\text{O}_2$  (BMS 3 Mk 2, PHM 71, Mk 2, Radiometer, Copenhagen). These parameters were kept normal during the experiments by means of artificial respiration.

Microspheres with a diameter of  $15 \pm 5 \mu\text{m}$ , labelled with  $^{141}\text{Ce}$  or  $^{51}\text{Cr}$ , suspended in 10% dextran were used (3M Company, St Paul, Minnesota). The microsphere stock solutions were resuspended using a mixer immediately prior to aspirating 0.15–0.5 ml into a 1 ml syringe. The syringe was rapidly attached to the three way stopcock on the arterial catheter and the injection was performed immediately. The injection syringe and catheter were flushed with 0.5 ml of 10% dextran in a second syringe previously attached to the stopcock. At the start of the injection venous blood from the nose and arterial reference samples were collected in 3 ml vials during 1½–2 min. Blood flow was measured as drops/min and the collected samples were later weighed. After 5–10 min the procedure was repeated with a differently labelled bolus of microspheres. In each cat either  $^{51}\text{Cr}$  or  $^{141}\text{Ce}$  labelled microspheres were injected once in order to investigate the regional distribution of capillary blood flow within the nasal mucosa, while the other isotope was injected 3–4 times when only arterio-venous shunting was measured.

After each experiment the maxilloturbinal (inferior concha) and ethmoturbinal (middle concha) on each side was removed and weighed. The separate blood and tissue samples were counted for 10 min in a gamma ray spectrometer (Packard Tricarb gamma spectrometer 3320).

The radioactivity in the arterial ( $A_{\text{counts ml}^{-1}}$ ) and venous ( $V_{\text{counts ml}^{-1}}$ ) blood samples measured. The percentage shunt

posterior part of the nasal cavity (Dawes & Pritchard, 1953), was exposed by a transorbital approach. To gain access, the eyelids were removed and the eyeball opened and emptied. The sphenopalatine vein was isolated from communicating veins and cannulated retrogradely from the deep facial vein in the angle of the mouth, using a polyethylene tube (PE 160). The tip of the cannula was positioned at the level of the nose. Reference arterial blood samples were collected from the right subclavian artery, which was cannulated with a polyethylene tube (PE 160). During the measurements the blood flow from the artery was adjusted by a clamp to a flow similar to that measured from the sphenopalatine vein. Heparin (Vitrum) 1000 IU/kg was given i.v. every second hour. Blood was re-infused into the left cephalic vein.

For the injection of the microspheres into the blood stream a catheter (PE 90) supplied with a three way stopcock was introduced into the brachiocephalic trunk (4 cats) or positioned in the ascending aorta (4 cats) via the right femoral artery. The positions of the catheters were confirmed by dissection at the end of the experiment. As it was found difficult to determine the position of the catheter within the aortic arch or brach-

ing) and shunt flow were then calculated according to the following equations

$$\% \text{ shunting} = \frac{100 \times V_{\text{counts ml min}}}{A_{\text{counts ml min}}} \quad (1)$$

$$\text{shunt flow} = \text{Total flow} \times \% \text{ shunting} \quad (2)$$

Capillary flow ( $C$ ) was then determined as the difference

$$\text{Capillary flow} = \text{Total flow} - \text{shunt flow}$$

The radioactivity (counts/g) in the tissue samples ( $T_{\text{counts g}}$ ) was also measured and regional capillary flow ( $C_i$ ) calculated as follows

$$C_i = \frac{T_{\text{counts g}}}{A_{\text{counts ml min}}}$$

## RESULTS

A large proportion of the microspheres passed through the nasal vascular bed in all experiments, indicating the presence of shunt flow. When the spheres were injected into the aortic arch or the brachiocephalic trunk the number of spheres passing through the microcirculation varied considerably between the different animals ( $\bar{X} = 44 \pm 24\%$ ,  $n = 15$ ). As it was found to determine the position of the injection catheter within the aortic arch or brachiocephalic trunk with certainty, the left atrium was cannulated in later experiments. This resulted in smaller variations in results between and within the different animals and a higher proportion of spheres passed through the microcirculation, when compared with results from the previous injection method ( $\bar{X} = 57 \pm 11\%$ ,  $n = 20$ ,  $p < 0.05$ ). When the number of microspheres in each injection bolus did not exceed 1.5 million injections could be repeated 3–4 times without noticeable reductions in blood flow from the sphenopalatine vein.

In 5 cats sympathetic stimulation regularly resulted in a decrease in venous outflow, accompanied by a reduction in shunt and capillary flow (Fig. 2). At a frequency of 0.5 imp/sec the reduction in shunt flow from resting values ( $\bar{X}_s = 77 \pm 9\%$ ,  $n = 5$ ) was higher than the reduction in capillary flow ( $\bar{X}_c = 28 \pm 14\%$ ,  $n = 5$ ,

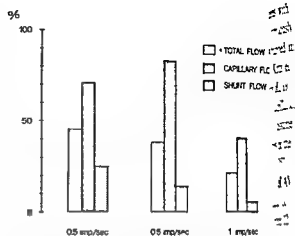


Fig. 2 The reduction from resting values in total, capillary and shunt blood flow in nasal tissues following sympathetic stimulation at 0.5 and 1.0 imp/sec. Resting flow = 100%. (Cat 41 kg)

$p < 0.001$ ) or total flow ( $\bar{X}_t = 56 \pm 9\%$ ,  $n = 5$ ,  $p < 0.02$ ).

In 6 cats regional capillary flow within the maxilloturbinal and ethmoturbinal on each side was established. To obtain sufficient counting accuracy, when counting the radioactivity from the trapped microspheres in the small amount of tissue of the maxilloturbinal, the number of microspheres in each injection had to be increased (about 2.5–4 million microspheres were required). It should be observed, however, that in addition to nasal mucosa the excised maxilloturbinal and ethmoturbinal also contain nasal cartilage and bone tissue. The capillary flow, expressed in ml/g/min, varied considerably in the different maxilloturbinal ( $\bar{X} = 0.61 \pm 0.12$ ), ethmoturbinal ( $\bar{X} = 0.51 \pm 0.43$ ), ( $\bar{X} = 0.41 \pm 0.36$ ,  $n = 8$ ), ethmoturbinal ( $\bar{X} = 0.41$ ,  $n = 8$ ). No significant difference in capillary flow was found between the maxilloturbinal and the ethmoturbinal when both sides were compared.

## DISCUSSION

In the present study, regional capillary flow was recorded by simultaneous measurement of venous outflow and the 15  $\mu\text{m}$  spheres method in cats.

Microspheres with a diameter greater than  $10\text{ }\mu\text{m}$  have previously been shown to be almost completely trapped in the microcirculation of the kidney (Katz et al., 1971) and the heart (Buckberg et al., 1971), indicating that they do not pass the capillaries within these organs. It is reasonable to assume that they also fail to pass the capillaries in the nasal vascular bed. Dawes & Prichard (1953) demonstrated and measured the diameter of AVA in neoprene casts of the nasal vascular bed of the cat. They observed diameters from  $12\text{--}60\text{ }\mu\text{m}$ , which indicates that the microspheres used in the present experiment are suitable for the study of AVA and capillary blood flow in the nasal vascular bed.

The present results which show that about 60% of the spheres passed through the nasal vascular bed, might seem very high. Methodological errors affecting the microsphere injection technique have been analysed by Buckberg et al. (1971) and their suggestion for reducing these errors have been followed in the present study. Thus the number of microspheres in each blood sample greatly exceeded 1000, making random variations in the distribution of the microspheres of minor importance. Thus the difference in results obtained following injections into the aortic arch or brachiocephalic trunk and the left atrium, respectively, is probably due to incomplete mixing of the spheres at the junction of the subclavian and common carotid arteries following the first type of injection. This implies that the injection should preferably be performed into the left atrium. The present results on shunt flow thus represent the functional consequences of the rich abundance of AVA in the nasal mucosa as described in morphological studies (Dawes & Prichard 1953; Rossatti 1954; Cauna 1970).

Labelled microspheres have previously been used to estimate blood flow in otorhinologic tissues including the nasal mucosa (Abe & Jackson, 1972). However the present finding that more than 50% of the spheres appeared to pass through AVA invalidate any calculations of total nasal blood flow with this technique and implies that the regional distribution of

spheres in the nasal mucosa will solely reflect the capillary flow.

When capillary flow was investigated in this way no clearcut difference between the two nasal cavities, the ethmoturbinal or maxilloturbinal area could be found. This indicates that the microcirculation was unaffected by the operation procedures on the right side. Capillary flow averaged  $0.5\text{ ml/g/min}$ , which is in agreement with previous results in the dog (Clairmont et al., 1973).

Morphological studies of the nasal mucosa have indicated an adrenergic, as well as a cholinergic, innervation of the arterial section of the AVA (Cauna, 1970). The difference in the relative response between AVA and capillary flow to sympathetic stimulation found in the present experiments indicates that AVA in the nasal microcirculation reacts in a similar way to the AVA in the skin and paw of the dog, where AVA responded more to  $\alpha$  receptor stimulation and blockade than did the capillary section (Spence et al., 1972).

Thus the present study provides evidence of an unusually abundant shunt flow in the nasal vascular bed and indicates that increases in sympathetic nerve discharge will result in a redistribution of flow from shunt vessels to exchange vessels.

## ZUSAMMENFASSUNG

Unter Verwendung von radioaktiven Mikrosphären ( $15\text{--}5\text{ }\mu\text{m}$ ) wurde die Kapillar- und Shuntblutzufluhr an der Nasenschleimhaut der Kätze bestimmt. Die Kapillarblutzufluhr betrug etwa  $0.5\text{ ml/g/min}$ . Gleichzeitig wurde eine reichliche arterio-venöse Shuntblutzufluhr registriert.

Die Shuntblutzufluhr betrug  $57\text{--}11\%$  der gesamten Blutzufluhr. Sympathische Stimulation fuhrte zu einer starken Verminderung der Shuntzufluhr als der Kapillazufluhr. Diese Befunde deuten darauf hin, dass eine Zunahme der sympathischen Nervenaktivität eine Neuverteilung der Blutzufluhr von den Shuntgefäßen zu den Austauschgefäßen zur Folge hat.

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## TOMOGRAPHIC EXAMINATION OF THE VERTICAL PART OF THE FACIAL CANAL IN CASES OF BELL'S PALSY

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(Received February 16, 1974)

**Abstract** In 41 patients with Bell's palsy of different degrees of severity a tomographic examination has been made of the vertical segment of the aqueduct of Fallopius from the second elbow to the stylomastoid foramen. The width and length of this segment of the canal and the occurrence of mastoid cellulae around the canal displayed large variations. There were, however, no significant differences between the affected and the sound side in the individual subject. A comparison between the findings in the palsy group and those in 30 healthy controls disclosed no significant differences in canal anatomy. The results of the study suggests that variations in the anatomy of the vertical segment of the canal are of only minor importance, if any, for the occurrence of Bell's palsy or for the extent of the damage to the nerve.

The cause of Bell's palsy is unknown. According to the most quoted of the theories on the background of the disorder the precipitating factor is ischaemia followed by impairment of the circulation within the facial nerve (Hilger, 1949, Kettel, 1959, Jain & Sharma, 1964, McGovern et al., 1972). It is considered that ischaemia in the nerve can arise through exposure to cold or inflammation (Collier, 1951, Kettel, 1959, 1963, McGovern et al., 1963), but also as a result of intravascular interruption of the nerve's venous supply (Fowler Jr, 1963). However, in facial palsy of the zoster-type, Devness (1968) found evidence that oedema and pressure were of subordinate importance.

Because of oedema, which is caused by, or which causes, ischaemia, the course of the nerve through a narrow rigid canal in the bone would worsen the paralysis, and also the prognosis.

Primary oedema is regarded as a cause of, and an aggravating factor in, ischaemia by Langworth & Taverner (1963), while Blatt & Freeman (1966) found oedema and inflammation in Bell's palsy unaccompanied by ischaemia. According to Hilger (1949), Sullivan & Smith (1950) and Kettel (1959) primary ischaemia, which they found usually to be located in the vicinity of the stylomastoid foramen, results in venous stasis and oedema, this is followed by secondary ischaemia, the collaterals in the bone canal then being compressed, thereby causing further impairment of the circulation. There is no objective proof that the canal lumen is a factor of importance in the development of the paralysis.

In conformity with the above hypothesis decompression of the canal to relieve pressure on the facial nerve is performed as a treatment for peripheral idiopathic facial palsy (Ballance & Duel, 1932, Sullivan & Smith, 1950, Jongkees, 1972, Pulec, 1972a). The value of, and particularly the indications for, this form of therapy have, however, been questioned by several researchers (Blatt, 1965, Miller, 1967, May & Hawkins, 1972, Diamant et al., 1972, Haglund, 1972), as conservative treatment has led to recovery of nerve function in 80-91% of the cases (Hilger, 1949, Laumans & Jongk 1963, Langworth & Taverner, 1963, Ha 1972).

According to neurophysiological



usual site of the paresis in Bell's palsy is the segment of the nerve from the geniculate ganglion to the stylomastoid foramen (Jepsen, 1965, Zilstorff-Pedersen, 1965, Jongkees, 1965, May, 1970). Even in subjects with normal function of the facial nerve individual deviations in the course of the nerve and considerable variations in the diameter of the canal lumen and the length of the segment have been observed in this segment of the canal (Kettel, 1959, 1963, Fowler, 1961, Michlke, 1965, Ericson & Liliequist, 1973). It is not known whether the size of the canal lumen in persons with Bell's palsy lies outside the normal range or whether the anatomy of the canal differs in subjects with persistent paralysis from that in persons who recover completely and rapidly, nor is it known whether there are such anatomic differences between the paralysed and the sound side in the same individual. With the object of throwing some light on these obscure points we have carried out a study of the course of the vertical segment of the facial canal in subjects with Bell's palsy of different degrees of severity.

### CASE SERIES

The study was performed on 41 subjects—16 men and 25 women—aged from 15 to 81 years admitted to the Department of Otorhinolaryngology, University Hospital, Umeå, with a diagnosis of Bell's palsy (peripheral idiopathic facial palsy).

The patients were submitted to (1) a clinical examination, (2) tests of chorda tympani function with determinations of taste and salivary secretion, (3) neurophysiological tests including EMG, and latency and threshold determinations.

#### (1) Clinical examination

Symptoms were registered and an evaluation of the paralysis in the three main branches of the facial nerve was made.

#### (2) Chorda tympani function

(a) *Salivary gland function* The function of the secretory fibres of the chorda tympani was tested by a method devised by Wiberg (1971).

(b) *Taste function* The function of the afferent fibres of the chorda tympani was examined by electrogustometry by Kraarup's method and by semiquantitative threshold determinations by a modification of Bornstein's method (Wiberg, 1971).

#### (3) Neurophysiological tests

Electromyography (EMG) was performed to examine the motor innervation of the mimic muscles, latency and threshold measurements were performed by routine methods in the Neurophysiological Laboratory.

The results of the above examinations were used in a four-grade classification of the series according to Diamant et al (1972).

*Group I* Complete recovery in 3 months

*Group II* Complete recovery in 3–12 months

*Group III* Incomplete recovery after 12 months, normal facial appearance at rest but defective function in spontaneous movements

*Group IV* Incomplete recovery after 12 months, paralysis obvious at rest and/or marked synkinesis, and/or marked loss of motor units as seen in EMG 12 months after onset of paralysis.

The sex distribution for group I+II and group III+IV is shown in Table I.

The palsy series was compared with a control group consisting of Umeå University students of both sexes with no history of paralysis or chronic ear disease. A detailed description of the control group has been given in an earlier paper (Ericson & Liliequist, 1973).

Table I Sex distribution of 41 patients with Bell's palsy divided according to severity of the nerve involvement

	Men	Women	Total
Slight-moderate (Group I+II)	12	17	29
Severe total (Group III+IV)	4	8	12
Total	16	25	41

*Tomographic examination*

The vertical segment of the facial canal from the outer bend on the second elbow to the stylomastoid foramen was examined by tomography in lateral projection by the method described by Ericson & Liljequist (1973). After deciding the depth of the section in preliminary tomograms the definitive examination was planned. This consisted of 5 tomographic sections parallel to the sagittal plane at intervals of 1 mm. An example of a tomographic cut through the vertical segment of the canal is given in Fig 1a. There was an enlargement factor of 1.3 due to the ray geometry. The films were taken with a Polytome tomograph, and the hypocycloid motion of the apparatus was used.

The tomogram on which the vertical segment of the facial canal was most distinctly visualized and on which the calibre of the canal was also widest was used as a basis for the measurements. The contour of the canal was marked with a sharp pencil, and the lumen was measured at the most proximal level (A), midway along the segment (B), at a point (C) midway between B and the stylomastoid foramen (D). The length of the segment A-D was also measured. The distances recorded are shown in Fig 1b.

All the measurements were performed by one observer with a magnifying glass bearing a scale graduated in tenths of a millimetre.

The occurrence of mastoid cellules along the canal lumen was also graded according to the scale: none (0), moderate number (1) and numerous (2).

Table II Means and standard deviation for the dimensions of the vertical segment of the aqueduct of Fallopius measured on lateral tomograms in 41 patients with Bell's palsy, millimetres

	Affected side		Sound side		Level of significance
	Mean	S.D.	Mean	S.D.	
Width of lumen at					
A	1.76	0.49	1.80	0.41	Not significant
B	2.10	0.44	2.15	0.48	Not significant
C	2.34	0.53	2.40	0.50	Not significant
D	3.17	0.92	3.18	0.75	Not significant
Minimum width	1.73	0.49	1.80	0.40	Not significant
Length of segment	16.07	2.31	15.85	2.08	Not significant

*Analysis of the tomographic method*

Analyses of the tomographic visualization of anatomic details in the temporal bone and of the errors ascribable to the identification of reference points and the measurements of the distances with the technique used have been reported elsewhere (Eckerdal, 1971, 1973, Ericson & Liljequist, 1973). The error of the method was small and negligible for this comparative study.

*Statistical analysis*

The calculations were performed on computers, and the usual statistical principles were used. Tests of parametric variables were carried out by Student's *t* test and of nonparametric variables by the  $\chi^2$  analysis.

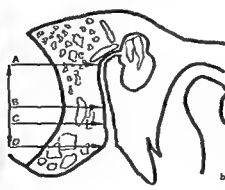


Fig 1 (a) Tomographic visualization of the vertical segment of the aqueduct of Fallopius from the second elbow to the stylomastoid foramen. Palsy. (b) The vertical segment of the canal showing points A, B, C, distance A-D.

Table III Mean difference (mm), standard deviation of the mean difference and coefficients of correlation between the affected and the sound side in the same subject for dimensions of the vertical segment of the aqueduct of Fallopius

Width of lumen at point												Length of segment		
A			B			C			D					
d	SD	r	d	SD	r	d	SD	r	d	SD	r	d	SD	r
-0.01	0.25	0.66***	0.01	0.40	0.40**	-0.07	0.55	0.19	-0.01	0.67	0.61***	0.27	1.43	0.79**

## RESULTS

The results are reported in Tables II-VII and Figs 1-4

The length of the vertical segment (A-D) of the facial canal, the size of the canal lumen in the sagittal direction for the levels, A, B, C and D and for the narrowest part of the lumen found empirically in the section, for both the paralysed and sound side, are given in Table II. The means and standard deviation for the variables were numerically equal on the two sides. As is seen from the standard deviation and Figs 1-4, however, there were large variations in the canal lumen and in the length and also the orientation of the canal in the sagittal plane.

To examine more closely whether there was any intra-subject difference between the paralysed and the sound side, the mean difference and the coefficient of correlation between the two sides were computed (Table III). The mean differences in the calibre of the canal between the paralysed and the sound side were small at the four levels of measurements. For the length of the vertical segment the difference was slightly larger but not statistically significant. As the standard deviations and correlation coefficients show, the differences between the sides in the individual subject could be quite large.

To examine whether variations in the severity of the paralysis might be due to differences in canal size the mean diameter of the canal lumen at different levels and the mean length of the canal segment were calculated for subjects in

group I and in groups III-IV, as was the mean intra subject difference between the two sides for groups I and III-IV. There was no statistically significant difference between subjects of these two groups as regards the canal parameters examined (Tables IV and V).

A comparison was made of the lumen size and the length of the vertical segment in the palsy and control groups (Table VI). The only significant difference was in respect of the lumen diameter at the stylomastoid foramen, which was significantly wider in the controls. This difference was found only in the men (Table VI).

Because of the large individual variations it was examined in analogy with Table IV, whether there was any difference in the canal anatomy between, on the one hand, the controls and on the other, mild and severe grades of palsy.

Table IV Means and standard deviations for the width and length of the vertical segment of the aqueduct of Fallopius in 17 patients with Bell's palsy group I and 12 patients in groups III+IV millimetres

	Group I		Groups III+IV		Level of significance
	Mean	SD	Mean	SD	
Width of lumen at					
A	1.84	0.36	1.87	0.34	Not significant
B	2.28	0.56	2.27	0.44	Not significant
C	2.50	0.60	2.53	0.50	Not significant
D	3.28	0.65	3.07	0.77	Not significant
Length of segment	16.00	1.60	15.93	2.57	Not significant

Table V Mean difference between affected and sound side in 17 and 12 patients with unilateral peripheral Bell's palsy group I and III+IV

	Width of lumen $\mu$								Length of segment	
	A		B		C		D			
	d	S D	d	S D	d	S D	d	S D	d	S D
Group I	-0.02	0.28	0.23	0.83	-0.12	0.60	-0.08	0.55	0.22	0.86
Groups III + IV	-0.01	0.40	-0.19	0.92	0.07	0.35	0.09	0.73	0.61	1.69

There were no significant differences between these groups except as regards the canal orifice (Table VII). In this comparison too, the difference was limited to the male subjects.

Comparison of the occurrence of mastoid cells around the vertical segment of the facial canal within the palsy group and between this and the control group disclosed no difference in either case.

## DISCUSSION

According to the tomographic examination of the vertical segment of the aqueduct of Fallopius the size of the lumen and the length and orientation of the canal in the sagittal plane varied fairly widely in the patients with Bell's palsy of different degrees of severity, on both the affected and the sound sides. This is particularly

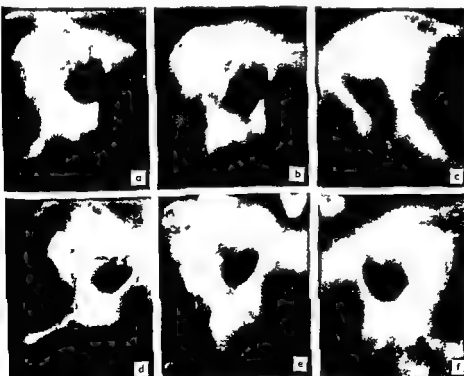


Fig. 2. Tomograms of the vertical segment of the aqueduct of Fallopius in 11 patients with unilateral peripheral Bell's palsy: (a-c) Palsy group I; (d-f) Palsy group III+IV.

Table VI Comparison between 41 patients with peripheral Bell's palsy and 30 healthy controls with the aqueduct of Fallopius

	Both sexes				Level of significance	Men				Level of significance
	Palsy		Controls			Palsy		Controls		
	r	S D	r	S D		r	S D	r	S D	
Lumen width at A	1.76	0.48	1.93	0.53	Not sign	1.83	0.50	2.06	0.42	Not sign
B	2.10	0.43	2.07	0.37	Not sign	2.06	0.40	2.18	0.38	Not sign
C	2.34	0.52	2.36	0.61	Not sign	2.33	0.58	2.59	0.49	Not sign
D	3.17	0.91	3.89	1.29	** ( $r = -2.73$ )	3.22	0.92	4.24	1.26	** ( $r = -2.73$ )
Length of segment	16.07	2.28	16.60	3.25	Not sign	16.28	2.84	17.44	2.24	Not sign

true of the canal length and the aperture in the stylomastoid foramen. In practically all subjects, however, the lumen was narrowest at the top and increased steadily in width in the peripheral direction. The results are consistent with those of a corresponding study of healthy subjects (Ericson & Liliequist, 1973). Chouard et al. (1971) state that the diameter of the mastoid part of the facial canal is largely con-

stant, but that there is an important narrowing where the nerve leaves the canal at the stylomastoid foramen. This view was also advanced by Cawthorne (1946) and Sullivan & Smith (1950), but is not supported by the results of the present study or that of Lindeman (1960). It is possible that these authors misinterpreted the funnel shaped orifice in some subjects as a narrowing (Figs 2a, b, c and 3b, d, e). So far as we are aware there

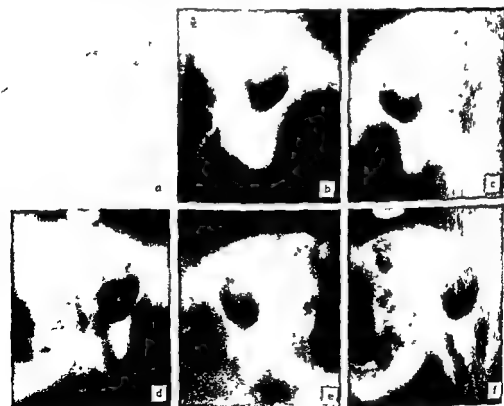


Fig. 3. Tomograms of the vertical segment of the aqueduct of Fallopius in patients with unilateral Bell's palsy. (a-c) Palsy group III. (d-f) Palsy group IV.

## respect to dimensions of the vertical segment of

Women					
Palsy		Controls		Level of significance	
x	SD	x	SD		
1.70	0.46	1.70	0.40	Not sign	
2.13	0.45	1.90	0.29	Not sign	
2.35	0.48	2.0	0.60	Not sign	
3.13	0.90	3.36	1.10	Not sign	
15.91	1.69	15.50	4.24	Not sign	

■ no previous study such as the present one or that performed on healthy subjects (Ericson & Liliequist, 1973)

With one exception no significant difference in the canal anatomy was observed in comparisons between the affected and sound sides, or between the affected side and the control group. The exceptional case concerns the variable for the stylomastoid foramen, which was significantly narrower in men with Bell's palsy than in the controls. Because this difference was bilateral and limited to this variable and one combination, the observation must be regarded as unreliable.

The tomographic examination of the vertical segment of the canal points fairly strongly to the fact that the variation in the width of the lumen and in the length and proximity of the canal segment to the mastoid cells in this segment itself has no bearing on the occurrence of Bell's palsy, or on the severity of the nerve

impairment. Whether the fact that the nerve passes through a rigid canal in the bone, and this alone, tends to cause palsy cannot, however, be ruled out on the basis of this study, since there may be co-variation between an aetiological factor and properties of the canal other than its anatomical shape. Nor can it be excluded that variations in other segments of the canal than the ones examined are of aetiological significance.

Because of the small number of subjects with severe palsy and because the quantitative grading of the paralysis was based on several variables, caution should be observed in evaluating the importance of variations in the anatomy of the canal as an aggravating factor.

We have observed that the horizontal part of the canal is narrower than the vertical segment, which gradually widens towards the stylomastoid foramen (cf Fig 4). That the width of the canal is not of primary aetiological significance is suggested also by the fact that oedema in the facial nerve in Bell's palsy is most extensive in the vertical segment (Pulec, 1972b), where we have found that the canal is widest. The observations that the nerve in the vertical segment takes up only about 35–50% of the canal lumen (James, 1961) and that the canal is narrower in the horizontal segment, irrespective of the fact that the lesions usually occur in the vertical part of the nerve (Jepsen,

Table VII Difference in the mean width of lumen and length of the vertical segment in 30 healthy controls and 29 patients with Bell's palsy in millimetres

Point	Difference in means				Length
	A	B	C	D	
Control Group I	0.14*	0.03*	0.04*	0.64*	0.60*
Control-Groups III-IV	0.20*	0.14*	0.09*	0.89*	0.33

\* None of the differences was significant



Fig 4 Tomograms of the vertical (a) and segments of the aqueduct of Fallopius horizontal (-) segment in narrower vertical segment

1965, Zilstorff-Pedersen, 1965, Jongkees, 1965, Miller, 1967, May, 1970), also suggest that the peculiarities in the canal's anatomy are not of critical importance for the occurrence of Bell's palsy and its prognosis. Conflicting with this view is the observation by Fisch & Esslen (1972) on exposing the canal that in 11 out of 12 cases of Bell's palsy the maximum nerve oedema was located between the geniculate ganglion and the porus acusticus internus.

Collier (1951) and Sunderland & Cossar (1953) found that the nerve occupied only 25–50% of the cross sectional area of the canal, and Lindeman (1960) gave a range of 10 to 50%. Hilger (1949) and James (1961) found that the nerve has a thick perineural capsule between the stylomastoid foramen and the stapedius nerve. This latter observation may be consistent with our experience that the canal lumen widens towards the orifice. The amount of connective tissue, the tension in the connective tissue around the nerve and the thickness of the nerve capsule in different sections of the canal might be of significance for the occurrence of palsy, and probably more important than anatomic peculiarities of the bony canal. Observations consistent with this have been reported by, among others, Hazama et al (1972).

## ZUSAMMENFASSUNG

Bei 41 Patienten mit idiopathischer Fazialislähmung (Bell's palsy) wurde der Fallopische Kanal vom zweiten Knie bis zum Foramen stylomastoideum mittels Tomographie untersucht. Die Untersuchung wies grosse Variationen in Weite und Länge dieses Kanalsegments und auch in der Menge der den Kanal umgebenden Mastoidzellen auf. Bei keinem Patienten existierte ein signifikanter Unterschied zwischen der paretischen und der normalen Seite. Auch zwischen den Patienten mit Lähmungen und 30 gesunden Kontrollpersonen gab es keine signifikante Unterschiede in der Kanal Anatomie. Die Resultate von diesen Untersuchungen deuten an dass Variationen in der Anatomie des vertikalen Kanalteils keine sichere Beziehung zum Verlauf und zur Prognose der idiopathischen Fazialislähmung haben.

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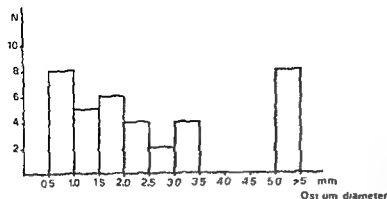


Fig 2 Histogram over the variations of the functional size of the maxillary ostium measured in 37 normal cases

6-7 mm and variations in length have a considerably smaller influence on the gas exchange through the ostium than variations of the diameter or cross sectional area. Furthermore, variations in the length cannot be determined in humans with the described method.

A possible complication might arise because of air embolism. However, the method was only used with subjects in whom the recordings have shown that the cannula was not lying in the mucosa and that the ostium was patent. The air insufflation lasted only a few seconds at most, only using different rates of airflow, beginning with the lowest, the pressure rise within the sinus did not exceed 3 cmH<sub>2</sub>O (2.20 mmHg). The risk of embolism was completely avoided by taking these precautions.

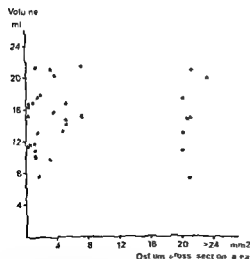


Fig 3 Relation between the volume of the maxillary sinus and the functional size of the maxillary ostium. No correlation was found.

The method cannot be used for sinuses with ostia larger than 5 mm in diameter, since the pressure increase even with 1 l/min airflow will be too small to be accurately recorded with our equipment. However, these cases are relatively few and it is usually sufficient to know that the ostium is larger than 5 mm in diameter since this size will facilitate an unrestricted gas exchange. The borderline value for relative insufficiency in the antral ventilation, indicated by a lower oxygen tension in the sinus, is at an ostial diameter of 2.5 mm (Aust & Drettner, 1974). It is thus of interest to note that the mean ostial size in this investigation was 2.4 mm, showing that the borderline for relative ostial insufficiency and the mean ostial diameter are almost identical.

## ZUSAMMENFASSUNG

Die Grösse des Ostiums wird durch die Kieferhöhle geschickt wird. Zu diesem Zweck wird der Luftstrom durch eine Kanüle in den unteren Nasengang in die Kieferhöhle geleitet und geht durch das Ostium hinaus. Die Druckerhöhung ist vom Luftstrom, der in den Sinus geleitet wird, und von der Grösse des Ostiums abhängig. Modellversuche wurden durchgeführt.

durchschnittliche funktionelle Ostiumgröße von 2.4 mm entspricht. Es gab keinen Unterschied zwischen Männern und Frauen in der Grösse des Ostiums und auch keine Korrelation zwischen dem Volumen der Kieferhöhle und der Grösse des Ostiums.

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## SCANNING ELECTRON MICROSCOPIC STUDIES OF NASAL POLYPS

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(Received March 25, 1974)

**Abstract** Nasal polyps yielded specimens, which technically were most suitable for study in the scanning electron microscope. A small number of glands and cytoplasm protuberances from the epithelial cells were found 30% of the total surface was covered by cilia. The cilia looked normal with no signs of destruction. In half of the specimens, areas with widened intercellular spaces were observed. The surface of nasal polyps did not differ essentially from that of the inferior turbinate in patients with perennial rhinitis. It was, however, characteristic of the polyps that cells from a transitional like epithelium protruded, dome like at the surface, resembling cobble-stones. Besides this a great variation of the surface area of the cells was found, with the occurrence of large cells with a surface area of  $p$  to  $150 \mu m^2$ . Another characteristic feature was the occurrence of an irregular surface with an appearance resembling a hilly country, in which every unevenness consisted of several cells.

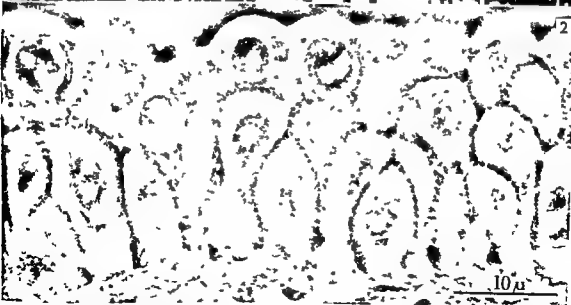
Nasal polyps are morphologically well defined structures, which represent the most extreme degree of oedema in the mucous membrane. The formation of nasal polyps may be due to allergy, but may also have other causes such as mucoviscidosis (Rulon et al, 1963). Extensive histological studies have been made (Andersen, 1943) and also two transmission electron microscopic studies of nasal polyps (Cauna et al, 1972, Jahnke, 1972), but as far as is known to the present authors the problem has not been systematically investigated with the scanning electron microscope (SEM). The ultrastructure of the surface of nasal polyps differs in certain regards from what we previously have described for the inferior turbinate in normals and in patients with perennial rhinitis (Mygind &

Bretlau, 1973 and 1974). We therefore find justified to publish our SEM studies of nasal polyps.

### MATERIAL AND METHODS

In 18 consecutive patients removal of a relatively free-hanging polyp was performed without local anaesthesia. The patients were allergologically examined, and allergy was demonstrated in 4 cases. None of the patients were treated with corticosteroids, locally or systemically. 13 specimens were examined by SEM and were embedded for microscopy in anoptal contrast.

The polyps were immediately fixed in a 5% solution of glutaraldehyde, buffered with 0.03 M sodium cacodylate (pH 7.4). The osmolarity of the solution was measured to 5 mOsm/l. After 24 hours of fixation the polyps were stored in a 0.15 M sodium cacodylate buffer (295 mOsm/l). After dehydration in ethyl alcohol the specimens for light microscopy were embedded in Epon. Sections were cut on a Reichert ultra-microtome UM 2 with a Dupon diamond knife. Semithin sections were analysed in anoptal contrast. The specimens for SEM were, after dehydration in ethyl alcohol transferred to benzene and freeze-dried by the method described in detail by Norrevang & Wirstrand (1970). The specimens were studied with a Cambridge Stereoscan S2.



*Fig 1* Typical surface of nasal polyp. The single cells which resemble cobble-stones are covered by short microvilli. The arrows indicate cells penetrating the epithelium probably eosinophil leucocytes  $\times 3270$

*Fig 2* Corresponds to Fig 1. A low epithelium of the transitional type with box formed surface-cells. It can just be seen that these cells have microvilli on the surface. Original magnification  $\times 630$

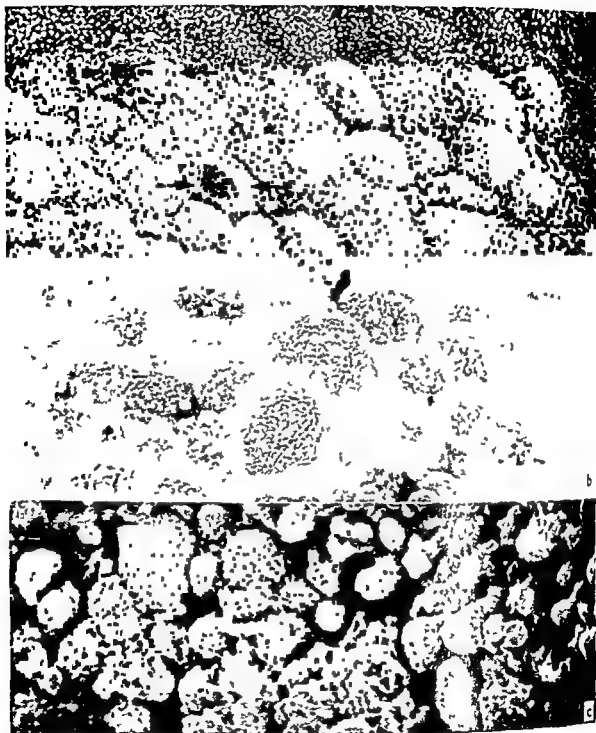


Fig 3a-c Various surface reliefs which can be found on nasal polyps as well as on the inferior turbinate. (a) The surface is even and covered by microvilli. Filled goblet cells can be identified by their nearly smooth surface (arrows). They may be surrounded by a ring of microvilli. (b) The cells are more prominent and the surface

irregular, but the terminal bars appear intact. The surface of some cells is granulated because the thin microvilli are retracted. (c) The surface is highly irregular and granulated. The cells are more prominent and the surface is highly irregular.



Fig 4 A more coarse irregular surface which relatively often occurs on nasal polyps. On the other hand, it is

unusual to see so many cytoplasm protuberances (arrows) from the epithelial cells  $\times 1680$

## RESULTS

The ultrastructure of the mucous membrane on the inferior turbinate has been described previously (Mygind & Bretlau, 1973 and 1974). In this paper we shall mainly describe structures which are characteristic of nasal polyps and regarding description of squamous epithelium, cilia, microvilli, goblet cells and other structures we must refer to our previous publications.

All polyps gave specimens fit for use. The epithelial surface was practically free of secretion, and only a few glandular openings were noted. The appearance of the surface differed considerably from one polyp to the other and from place

to place on the same polyp. Thus it could be observed that while the anterior surface of a polyp was covered entirely by microvilli, the posterior surface was totally covered by cilia.

The type of epithelium varied from a pseudostratified cylindrical, to a squamous type, with intermediate forms in between. Pseudostratified cylindrical epithelium was most frequent, and in only three SEM specimen could a typical squamous epithelium be observed. The epithelium of the polyps showed variation in thickness and cell composition in the same mucous membrane on the inferior turbinate. A characteristic low epithelium of intermediate type, resembling transitional



*Fig 5* Typical for nasal polyps, an extraordinarily great variation in the surface area of the epithelial cells, observed particularly in the presence of very large cells. The surface is covered by microvilli but a single group of cilia are seen, corresponding to one cell (arrow)  $\times 1680$

*Fig 6* Very occasionally, flat cells can be seen lying on top of the normal epithelial cells. This photo is from one of the rare glandular ducts, the surface is therefore covered with secretion. Original magnification  $\times 800$ .

found in the urinary bladder, was seen in several places. By means of SEM, the surface-cells in this epithelium appeared box-shaped and protruded, dome like at the surface, giving a general appearance of cobble stones (Figs 1 and 2). The surface relief with the individual cells can also vary considerably. Besides those cells looking like cobble-stones, it is also possible to observe an even surface, where the cells are found as flagstones in a mosaic (Fig 3a), or an irregular prominence of the individual cells (Fig 3b), or finally, a picture with defective terminal bars and widened intercellular spaces (Fig 3c). The last mentioned phenomenon could be seen in one or several places in half of the specimens. This corresponds approximately to the conditions on the inferior turbinate in patients with perennial rhinitis. The cells resembling cobble stones are, however, characteristic of polyps (Fig 1) and the occurrence of an irregular surface with an appearance resembling a hilly landscape in which every unevenness consists of several cells (Fig 4).

The cell borders are most often visible and slightly depressed. The surface shape of the cells is often round, but may be polygonal (Fig 3a). It is very characteristic to see a great variation in the surface area of the cells with the occurrence of large cells with a surface area of up to  $150 \mu\text{m}^2$  (Fig 5). Most of these cells are, in anoptical contrast, found to be the previously described large box shaped cells in a transitional epithelium, but in a few places it is possible in a typical pseudostratified cylindrical epithelium to see single large cells without contact with the basement membrane, lying on top of the normal epithelial cells (Fig 6).

Filled goblet cells could be identified, but cells being emptied were seldom seen. The same applied to cytoplasm protuberances from the epithelial cells (these formations are also called apocrine secretion) which were found in a considerably lower number than on the inferior turbinate. Now and then cells could be seen penetrating the epithelium (Fig 1). These cells are probably eosinophil leucocytes.

On the polyps, microvilli have the same ap-

pearance and are found to the same extent as on the inferior turbinate. The same applies to cilia, which cover about 30% of the total surface of the polyps. It should be noted that visible destruction of the cilia or signs of increased ciliogenesis was not observed in one single specimen. It is, however, impossible to say what the normal rate of ciliogenesis is in the mucous membrane in the upper part of the nasal cavity. We have not been able to collect suitable normal references from this area, but have only succeeded in obtaining two specimens from the upper part of the respiratory nasal mucosa in patients undergoing ethmoidectomy on account of sinusitis. In these specimens ciliogenesis was not observed. The surface was covered with normal-looking cilia, which appeared slightly longer than the cilia described at the anterior part of the inferior turbinate (Mygind & Bretlau, 1973). However, further investigations are needed to confirm this observation.

## DISCUSSION

Nasal polyps are most suitable for SEM in investigations, as a large untouched and secretion free surface can be obtained for microscopy without any kind of anaesthesia. However, it is important to choose a surface which has not been pressed against the mucous membrane of the nose or against another polyp. The microscopy is greatly facilitated because there is almost no mucus on the surface at all. This is undoubtedly due to the small number of glands, the contribution of gravity in removal of mucus and the influence of the inspired air on the mucous membrane, which is thereby transformed in the direction of the mucous membrane in the front of the nasal cavity.

The surface of nasal polyps does not differ essentially from the surface of the inferior turbinate in patients with perennial rhinitis. This has been described in previous publications (Mygind & Bretlau, 1973 and 1974) of the epithelium as the polypium may be the reason why.



of transitional type is more often seen in the polyps. The epithelial cells are often dome-shaped apically, which Lenz (1972) also has observed on the inferior turbinate in patients with perennial rhinitis. This phenomenon is most easily explained as a result of intracellular oedema. However, we have not observed the very pronounced degrees of intra- and intercellular oedema, as described in the transmission electron microscope study of Jahnke (1972). In half of the specimens the intercellular space between the epithelial cells was widened in a single part or in the whole of the biopsy. It must not be forgotten, however, that quite pronounced changes take place in a biological material when it is fixed, dehydrated, freeze-dried and metal-coated. If it was the case, *in vivo* too, that the intercellular spaces were greatly widened and the terminal bars quite defective, this would mean that the large amounts of tissue-fluid in general would be free to diffuse through the epithelium, as the terminal bars are the most effective barrier against such a transport (Ter-rahe & Backwinkel, 1970). It is not in accordance with common clinical experience to see

gross secretion of any importance from nasal polyps, since the latter would, in that case, spontaneously diminish. Several papers have unanimously shown that epithelial cells and pieces of epithelium are discharged in increased quantities in allergic mucous membranes (Mygind & Thomsen, 1973; Naylor, 1962; Sanerkin & Evans 1965). The most obvious explanation of the above-mentioned phenomenon is therefore that in the areas in question, a certain intercellular oedema is present as well as reduced cohesion between the cells. Shrinkage during preparation augments these tendencies and the specimens appear in SEM with greatly increased intercellular spaces and entirely defective terminal bars. The phenomenon observed must therefore be a structurally dependent artifact and, as such, a valuable observation.

The most striking new information obtained by the SEM investigation of nasal polyps, is that the surface-area of the epithelial cells can vary considerably. It is now known what dif-

ferences there may be between small and large cells regarding age and function.

It was an important observation that the cilia on nasal polyps had a quite normal appearance. It is therefore most likely that the destruction of cilia, which we previously have observed on the inferior turbinate in patients with perennial rhinitis, is due to the fact that these cilia are exposed to pressure from an increased amount of mucus or from the nasal septum, with which the swollen turbinate temporarily comes into contact. It may be concluded that in the chronic allergic mucous membrane, no signs are found of the specific destruction of cilia which has been described in sensitized animals, exposed to grass-pollen (Chevance, 1971).

The changes we have found on the surface of nasal polyps were of such a character that there is no reason to assume that they are specific of polyps of allergic genesis. This assumption is confirmed by the fact that it was impossible to detect any differences between the surface of the polyps from patients with positive, and from patients with negative allergological examination.

## ACKNOWLEDGEMENTS

The Cambridge Stereoscan microscope was kindly placed at our disposal by the Institute for Historical Geology and Palaeontology (H. J. Hansen M.Sc.). We owe Mr Jørgen Fuglsang great thanks for operating the microscope for us. Gratitude is also expressed to Mrs Bente Christensen and Mr Erik Jensenius for skilful technical assistance.

## ZUSAMMENFASSUNG

Nasenpolypen konnten gut im Rasterelektronenmikroskop untersucht werden. Es wurde eine geringe Anzahl Drüsen und Protuberanzen des Zytoplasmas der Epithelzellen gefunden. 30% der totalen Oberfläche war von Zilien ohne Anzeichen einer Destruktion bedeckt. Die Hälfte der Präparate wies Gebiete mit erweiterten Interzellularräumen auf. Bei Patienten mit vasomotorischer Rhinitis unterschied sich die Oberfläche der Polypen kaum von der der unteren Nasenmuschel. Es war aber charakteristisch, dass beiden Polypen Zellen eines Übergangsepithels wie Pflasterzellen kuppelförmig hervorragten. Ausserdem wurde bei Zellen bis zu einer Oberflächengrösse von 150 µm<sup>2</sup> eine grosse Variation des Oberflächeninhalts beobachtet. Eine andere charakteristische Erscheinung war eine un-

ebene Oberfläche, einer hügeligen Landschaft ähnlich, deren Unebenheiten jeweils aus mehreren Zellen bestanden

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## THE INFLUENCE OF TEMPERATURE ON MUCOCILIARY ACTIVITY

*Temperature range 20°C-40°C*

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(Received January 24 1974)

**Abstract** In vitro experiments have been performed on mucous membranes from rabbit tracheae in order to investigate the influence of temperatures between 20 and 40°C on the mucociliary activity

The following results have been obtained

1 The mucociliary activity including mucociliary wave frequency and coordination of wave patterns is most regular at or near the body temperature

2 From body temperature to room temperature a 50% reduction of the wave frequency was demonstrated together with irregular changes in frequency and amplitude

3 The effect of temperature alterations was reversible within at most 3 hours

4 The relationship between mucociliary wave movements and temperatures between 20° and 40°C looked practically linear contrary to most temperature dependent physiological processes This indicates that more than one factor is involved in the surface light reflections ■■ the intracellular activity and mucus rheology

Temperature influences all biological processes including the function of the mucociliary system of the respiratory tract Experimental investigations illustrating this subject have been published by many investigators using different methods The transport velocity in mammals has been studied by among others Dalhamn (1956), Hill (1957) and Tanaka (1967) These authors found a maximum effect of ciliary transport at a temperature of 36-40°C The ciliary beat frequency in mammals has been investigated by Ballenger & Orr (1963) and by Iravani (1967) using stroboscopy and also by Proetz (1934),

This investigation has been supported by grants from the Swedish Medical Research Council Project number B73 14X 3897-01

Dalhamn (1956, 1960) and Iravani & Steinhausen (1967) with the use of cinematographic methods (Table I) Tanaka (1967) has compared the ciliary beat frequency of rabbit tracheae at different temperatures in vivo by a photoelectric technique He found maximum ciliary function at about 30°C

The use of different experimental animals and a number of different recording techniques makes it impossible to compare the cited investigations Furthermore, a review of the literature failed to reveal any investigations based on a systematic study of graduated increases and decreases of the temperature parameter within the range of 20° to 40°C

The mucociliary system of the respiratory tract may be influenced by temperature changes during febrile illnesses and also as a result of hypothermia as utilized in special types of anaesthesia The ciliary function may also be influenced by the temperature of the inspired air if the physiological air conditioning of the nose is bypassed

In view of this clinical background we considered that a special survey of the temperature parameter might be of theoretical and practical importance Furthermore, it is necessary to know the effect of temperature changes on mucociliary activity in the temperature range between room temperature and body temperature, since many applied experimental studies on cilia are made on animals in the laboratory environment

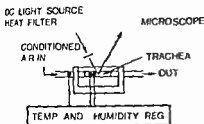


Fig 1 Experimental arrangement for indirect recording of mucociliary wave patterns via surface light reflections in an air-conditioned experimental chamber

## METHOD AND MATERIAL

A standardized method for the study of mucociliary wave movements has already been published (Mercke et al, 1974; Toremalm et al, 1974). Mucociliary wave movements are recorded indirectly via surface light reflections *in vitro*.

After thorough preparation, part of a rabbit trachea was mounted in a special experimental chamber, as illustrated in Fig 1. A light beam was directed onto the tracheal mucous membrane through a slit made in the pars membranacea. The variations of light intensity picked up by the microscope are transformed into electrical voltages and amplified by the photomultiplier and recorded by an ink writer. The temperature of the tracheal specimen and the air blown through it in one direction were monitored by four thermocouples. Two of them (ELLAB type KCl) were placed within the tracheal lumen for control of the air temperature and two on the outside of the tracheal wall.

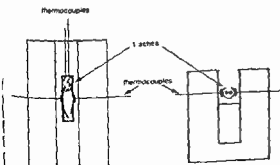


Fig 2 Cross section of the experimental perspex chamber containing the tracheal specimen. The sites of intra-tracheal and extratracheal thermocouples are shown.

(ELLAB type HI) for control of the tissue temperature, as seen in Fig 2.

The investigation was comprised of eleven experimental sequences with tracheal specimens from 6 rabbits with a weight of 1–1.5 kg. The animals were killed with a blow on the head in order to avoid pharmacological side effects. The relative humidity was kept between 90–100%, which was controlled by a psychrometer (ELLAB type B19).

The recordings were analysed and the average wave frequency determined for periods of 60 sec. In addition, the frequency spectrum of the mucociliary wave movements was analysed by computer.

## RESULTS

A summary of all results is given in Table II. The results from one experimental sequence are shown in Fig 3. A gradual increase of temperature from 21°C to nearly 40°C during 85 min resulted in an increase of the mucociliary wave movements from about 420 to about 1000 waves/min. Increased temperature also brought about a more uniform rhythm. With a subsequent decrease in temperature to 19.1°C during 197 min the frequency of the wave movements was reduced to 360 waves/min and the rhythm became more irregular again with a great number of variations in amplitude and frequency.

Fig 4 shows another experimental sequence in which the temperature was increased from 20 to 40°C followed by a subsequent decrease to 20.9°C. The temperature was then once again increased to 40.3°C. This experimental sequence lasted 313 minutes. A maximum uniformity of the mucociliary wave movements was observed at 35–40°C.

The results from three different temperature ranges in Fig 3 were also processed by computer (Fig 5). As the temperature increased from 20.9 to 39.9°C, the frequency of mucociliary wave movements also increased from 20 waves/sec to 20 waves/sec. The scatter is about 2 waves/sec at each of these temperatures.

All the results regarding tracheal temperature and mucociliary activity at various

Table I

S = Stroboscopical methods C = Cinematographical methods P = Photoelectrical method

Author	Method	Animal	In vitro	In vivo	Temp range (°C)	Freq range (beats/min)	Max frequency at (°C)
Proetz, 1934	C	Rabbit		×	5-50	0-720	18-33
	C	Homo	×		5-50	0-720	18-33
Dalhamn, 1956	C	Rat		×	37-41	1 097-1 290	41
Dalhamn, 1960	C	Rabbit		×	33.9-38.6	911-1 404	38.6
Iravani Steinhausen, 1967	C	Rat	×		4-25	10-264	—
Ballenger Orr, 1963	S	Guinea pig	×		C 20-38	850-1 600	—
Iravani, 1967	S	Rat	×		4-44	—	38-40
Tanaka, 1967	P	Rabbit		×	20-40	C 1 030-1 170	C 30

have also been plotted graphically. The frequencies of mucociliary wave movements were relatively well concentrated along a regression line (Fig. 6). The frequency/temperature relationship is not strictly logarithmic or linear. The regression line flattens out slightly at temperatures at and above body temperature.

### DISCUSSION

The mucociliary function of the respiratory tract is influenced by the body temperature and may also be affected by the temperature of the inspired air. This relationship has long ago been

observed by many investigators using stroboscopic, cinematographic or photoelectric methods (Table I). The results obtained during a period of four decades, vary greatly. Cilia from different mammalian tissues have been investigated experimentally under various degrees of control. The maximum frequency was found within a limit of 18° to 41°C. As far as we know there has been no investigation based on continuously increasing and decreasing temperatures between 20° and 40°C with satisfactory simultaneous control of the relative humidity of the surrounding air. It has not therefore been possible to draw any definitive conclusions from the

Table II

I = Temperature increment  
D = Temperature decrement

		Initial temperature (°C)	Final temperature (°C)	Experimental duration (min)	Mucociliary wave movements (waves/min)	Number of observations
Rabbit no. 1	D	40.4	21.1	165	1 034 → 292	16
Rabbit no. 2	I	21.0	40.3	85	420 → 1 020	11
	D	40.3	19.1	197	1 020 → 360	16
Rabbit no. 3	D	35.0	20.1	108	893 → 345	9
	I	20.1	38.3	72	345 → 1 007	8
Rabbit no. 4	I	23.8	40.9	87	428 → 751	10
	D	40.9	27.0	135	751 → 509	9
Rabbit no. 5	I	21.1	40.2	81	390 → 972	11
Rabbit no. 6	I	19.9	40.1	61	341 → 779	8
	D	40.1	20.9	191	779 → 295	10
	I	20.9	40.3	61	295 → 843	7

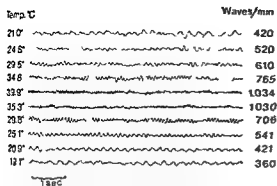


Fig. 3 Recordings and average mucociliary wave frequencies from one tracheal specimen at various temperature intervals between 19.1° and 39.9°C

literature quoted regarding the temperature influence on mucociliary activity in the respiratory tract

Our standardized method for experimental *in vitro* studies of cilia is described in a previous paper (Mercke et al., 1974). It is also necessary to standardize the temperature and humidity parameters during applied experiments, for example where respiratory cilia are used to test the influence of inhaled gases and particles. For laboratory purposes the temperature range between 20° and 40°C—representing ordinary room temperature to body temperature—is of most importance and interest.

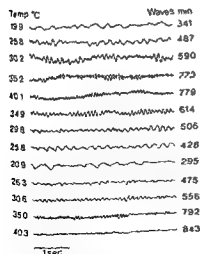


Fig. 4 Recordings and average mucociliary wave frequencies from one tracheal specimen at various temperature intervals between 19.9 and 40.3°C

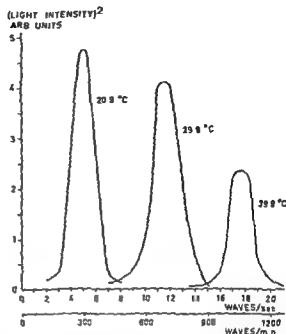


Fig. 5 The relationship between mucociliary wave frequency at three different temperatures and reflected light intensity in the sequence also shown at Fig. 3. At each temperature, the frequency varies by about  $\pm 1$  wave/sec.

In the recordings shown in Fig. 3 the mucociliary wave frequency is about 1000 waves/min at body temperature. The rectal temperature of afebrile rabbits is at most 39°C according to Altman & Dittmer (1966). In the present investigation we have found that a temperature reduction from 40° to 20°C is accompanied by a decrease of the mucociliary wave frequency of about 50%.

According to the very few actual experiments available (Table 1) the difference between *in vivo* and *in vitro* experiments seems to be about 200 waves/min. There are, however, many arguments in favour of *in vitro* experiments. For example, such disturbing factors as interference from heart- and respiratory movements and alternating air flow directions, as well as the influence of anaesthetics, can be excluded. However, the rhythmical contractions of the smooth muscles of the tracheal wall may also be observed in *in vitro* experiments (Håkansson & Tamm, 1967).

As can be seen from the surface r

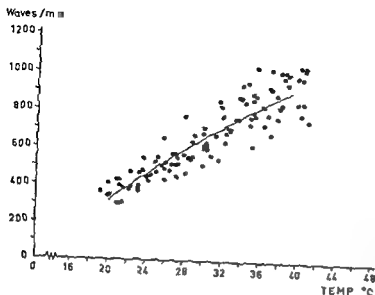


Fig 6 The relationship between all wave frequency averages and temperatures in the range 20–40°C (cf Table II) for the 11 experimental sequences

shown in Figs 3 and 4 the frequency and amplitude of the reflections are not the only points of interest. There is also a more or less regular rhythm of amplitude and frequency variations.

Since the reflections are dependent on mucous surface waves as well as on moving cilia (Tore malm et al., 1974) an incongruence may appear in the two reflection points. At 35–40°C

the rhythm is very regular which can be due to addition of the two reflections without any appreciable phase shifting. At lower temperatures (20–30°C) the cilia are presumed to work with a reduced continuous frequency and the mucous waves may be retarded due to increased tenacity. While the mucous ridges are lowered, giving less light reflection, the cilia are at the same time sweeping more slowly, giving an extended period of reflection. The recordings at 20°C are therefore irregular, in addition to the reduction of mucociliary wave frequency and decrease of secretional wave amplitudes.

The distributions of average wave frequencies at three temperatures from the sequence shown at Fig 3 have been processed by computer. The result appears at Fig 5. From this the mean frequency and scatter at each temperature can be easily seen. The scatter is only about  $\pm 1$  wave/sec at all three temperatures. This speaks in favour of a regular basic frequency produced by the cilia. The total number of results obtained

from the trachea of all six rabbits are shown in Table II. We have not found any signs of hysteresis in the sequences with successive increases and decreases of temperature.

All results have been pooled in a frequency/temperature diagram (Fig 6). From this it is obvious that the frequency of reflections increases with increasing temperature, following a regression which is virtually linear. The range of dispersion between the animals is about 100 waves/min at 20°C and 300 waves/min at 40°C. This curve differs from the usual pattern of biological processes, where a rise in temperature is generally combined with an exponential increase of activity. Guttman (1969) for example has shown increase in  $Q_{10}$  values for repetitive responses in squid axons following increased temperatures. For surface tension forces however, the temperature dependency is strictly linear (Adamson, 1967). In the present investigation the  $Q_{10}$  value was 2.1 between 20 and 30°C and 1.4 between 30 and 40°C. It is most plausible that more than one parameter is involved in the present results summarized in Fig 6. We know some of them. One is the intracellular "pacemaker", another the tenacity of the secretion and a third the surface tension. In the present case, the tenacity and surface tension forces are dominant at low temperatures, so that an exponential increase cannot

expected. It should therefore be very interesting to study the ciliary wave frequency without the mucous cover. However, such changes of the mucous layer are not possible without impairing physiological functions (Sade et al., 1970).

The present results do not allow any direct conclusions regarding the influence of temperature on mucociliary function from a clinical point of view. The difference between the function of human and mammalian respiratory cilia is very small (Proetz, 1934) as is also the difference between *in vivo* experiments and tracheal *in vitro* preparations taken immediately post mortem. A functional reduction of mucociliary activity is therefore probable e.g. in patients during hypothermic anaesthesia. A far more interesting problem is the mucociliary transport mechanism in febrile patients. The etiology of bronchopneumonia and atelectasis especially needs to be studied from this point of view. Regarding the possible influence on cilia of untemperated inspired air, there are no problems during physiological ventilation thanks to the excellent heat and moisture exchange function of the nose and larynx. It has been found that during nasal breathing at a room temperature of 21°–27°C the inspired air was 32–34°C when it passed the nasopharynx (Cole, 1953). Ingelstedt (1956) found a temperature of the inspired air in the subglottal space of 32.3°C during nasal breathing and 30.5°C during oral breathing. The difference is due to the intimate contact between the turbulent air stream and the mucous membrane made possible by the shape of the nasal cavities. Ingelstedt & Toremalm (1960) found an air temperature of 25°C in the nasopharynx of a patient exposed to an environmental temperature of 12°C. The inspired air is therefore well temperated when it passes through the trachea during normal breathing and does not greatly influence the mucociliary function.

The present basic laboratory study gave results which should be considered as showing that standardized recording methods are in themselves inadequate. The temperature para-

meter must be kept under control in applied studies into the influence of pharmacological and toxicological substances on mucous membranes.

## ZUSAMMENFASSUNG

Der Einfluss von Temperaturen zwischen 20 und 40°C auf die mukoziliäre Aktivität ist vermittels *in vitro* Versuchen an der Trachealschleimhaut von Kaninchen untersucht worden.

Es ergaben sich folgende Resultate:

- 1 Die mukoziliäre Aktivität einschliesslich der mukoziliären Wellenfrequenz und der Koordination des Wellenmusters ist am regelmässigsten bei oder in Nähe der Körpertemperatur.
- 2 Bei Senkung der Temperatur von Körpertemperatur auf Zimmertemperatur konnten eine 50prozentige Herabsetzung der Wellenfrequenz sowie unregelmässige Frequenz- und Amplitudenveränderungen festgestellt werden.
- 3 Der durch Temperaturveränderungen hervorgerufene Effekt war innerhalb von höchstens drei Stunden reversibel.
- 4 Im Gegensatz zu den meisten temperaturabhängigen physiologischen Prozessen erwies sich das Verhältnis zwischen den mukoziliären Wellenbewegungen und Schwankungen der Temperatur zwischen 20 und 40°C als fast linear. Das Resultat deutet darauf hin, dass mehr als ein Faktor die Lichtreflexe an der Schleimhautoberfläche hervorruft, z.B. die Intrazelluläraktivität und Schleimrheologie.

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## THYROPLASTY AS A NEW PHONOSURGICAL TECHNIQUE

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(Received February 13, 1974)

**Abstract** In an attempt to examine the surgical possibility of changing the vocal cord position and tension by removing the thyroid cartilage, an experimental study was made using 10 adult dogs. Hoarseness produced by sec-

tion. Their effects on the vocal cord are (1) lateral compression, (2) lateral expansion (3) relaxation (shortening) and (4) stretching (lengthening) respectively. Possible indications for each type of thyroplasty were defined with reference to specific laryngeal diseases. The advantages of thyroplasty were emphasized namely, that an intervention inside the thyroid cartilage is minimal and therefore fine and reliable adjustment is possible during surgery. Thyroplasty thus offers a new possibility of phonosurgery.

The aim of treatment for laryngeal diseases is most frequently to attain a normal voice, although this becomes secondary in such cases as laryngeal carcinoma or bilateral recurrent nerve paralysis. Phonosurgery, defined as surgery for better voice, is at present almost limited to removal of a benign tumor on the vocal cord or reduction of the glottal chink by teflon injection or cartilage implantation for unilateral recurrent nerve paralysis. Despite the recent development of laryngo-microsurgical technique, direct intervention on the vocal cord itself, e.g. plasty for sulcus vocalis for instance, has not yet been performed.

There may be several factors which prevent direct surgical intervention on the vocal cord. Surgical techniques which can be used through a suspension laryngoscope are naturally limited and fine suturing as is necessary for a plasty is virtually impossible. Laryngofissure, which

would then be required for such a technique, poses another unsolved problem—postoperative development of granuloma at the anterior commissure. The greatest obstacle we encounter, however, is the inevitable postoperative scarring of the mucosa, which reduces the compliance of the vocal cord mucosa, thus greatly hindering vibration of the vocal cord.

As a result, phonosurgery at present is quite incompetent for laryngeal diseases such as mutational voice disorders, hyperfunctional dysphonia, vocal cord hypertrophy due to anabolic hormones, sulcus vocalis, vocal cord atrophy, and traumatic laryngeal deformities. We laryngologists should thus break away from conventional vocal cord polypotomy. As a stage prior to direct surgical intervention on the vocal cord, we attempted to change the position and physical property of the vocal cord by actively reforming the cartilaginous frame-work on which vocal cords are suspended.

A search of the literature fails to reveal any similar systematic investigation on how the reformation of the thyroid cartilage affects the vocal cord and the voice. This preliminary report deals with the experimental results of thyroplasty in dogs. Several clinical cases will be reported in detail later.

### EXPERIMENTAL PROCEDURE

Ten adult dogs were used for the experiment. The dogs were anesthetized by intraperitoneal injection of Pentobarbital Natrium (Mitsubishi) and fixed supine for laryngeal

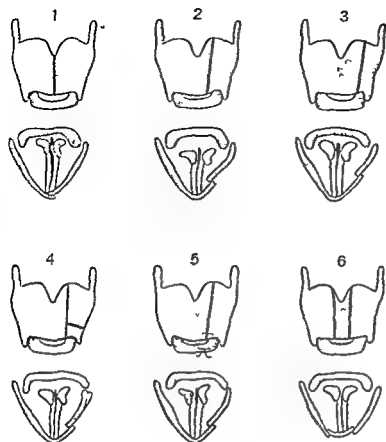


Fig 1 Schematic demonstration of varied thyroplasties performed on dogs

Local anesthesia with 1% Xylocaine, the right and left recurrent laryngeal nerve were exposed. For better visualization of the vocal cord, supraglottic laryngectomy was performed. Voicing was evoked by pain stimuli. For each experimental condition mentioned below, laryngeal pictures—black and white, color photographs, and 16 mm cinefilm—were taken during respiration and phonation. The voice was recorded by tape-recorder for later acoustic analysis. Before sectioning of the recurrent laryngeal nerve, the vocal cord movement and voice were confirmed to be normal. Any dog whose respiratory movement of the vocal cord was asymmetrical was excluded from the analysis of the experimental results.

After section of the left recurrent laryngeal nerve, thyroplasty was performed: vertical incision of the left lamina was made at 1 the median line, 2 anterior one third, 3 middle, and the lateral part of the cartilage lamina was

slipped underneath the medial part and fixed by 4-0 Nylon suture. The two laminae were overlapped 2-4 mm width depending on the case. In addition to the recurrent laryngeal nerve the external branch of the left superior laryngeal nerve was also cut in two dogs. Two parallel vertical incisions on the thyroid cartilage and depression of the median cartilage were done in one dog. Incisions and fixation of the thyroid cartilage used in the present experiment are schematically shown in Fig 1.

## EXPERIMENTAL RESULTS

### 1 Median incision on thyroid cartilage and repositioning of left lamina (1 in Fig 1, Dog no 1)

The hoarse voice caused by section of the left recurrent laryngeal nerve was somewhat improved by the above procedure but still re-



2 Left control, during phonation, middle after repositioning of the left recurrent laryngeal nerve, during

phonation, right after thyroplasty during phonation. The arrow indicates the site of repositioning

uned rough. The procedure resulted in the shift of the paralysed vocal cord slightly toward the midline, with the decrease of glottal slit during phonation. Nevertheless a noticeable ank remained at the posterior glottis (cartilaginous portion). The voice was lowered in pitch slipping in the left lamina.

2 Vertical incision of left lamina at anterior middle third and shift of lateral (posterior) lamina underneath the median (anterior) lamina (2 in Fig 1, Dogs 2, 4, 7, 8, 9, 10)

Hoarseness due to recurrent nerve section was markedly improved, particularly in dogs 4 and 9, whose voices became quite normal (Fig 1). In general, there remained a narrow slit at the cartilaginous portion. The vocal pitch did not change markedly. In dog no 8, the inner perichondrium was separated over a wide area from the thyroid lamina along the vertical incision line so that the lateral part could be slipped anteriorly underneath the median part. This wide perichondrium separation and overlapping of the laminae resulted in prominent bulging of the vocal cord, and the voice remained hoarse and low-pitched. The excessive increase in thickness seemed to have arrested the vibration of the vocal cord.

3 Vertical incision at antero-posterior mid line and shift of lateral part (3 in Fig 1, Dog no 3)

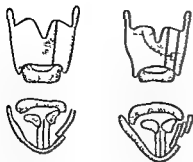
The hoarseness was not much improved by this procedure. A slit remained not only at the cartilaginous portion but also at the anterior glottis.

4 Vertical incision at anterior middle third supplemented by horizontal incision and shift of upper lateral part (4 in Fig 1, Dog no 2)

A slit always remained at the cartilaginous portion even following the procedures 1-3 in Fig 1. It was assumed that the inferior horn or cricothyroid joint was in the way of the lateral lamina shifting medially. For this reason, an additional incision was placed horizontally above (cranially) the cricothyroid joint to shift the posterior part of the lamina more medially.

This tilted T-shaped incision (1-4) unexpectedly ineffectuated the function of the cricothyroid muscle, because the muscle attachment on the thyroid cartilage was indirectly disconnected with the anterior commissure. Consequently the vocal cord was shortened and slightly abducted. The slit did not become narrower and the resultant voice improvement was not remarkable.

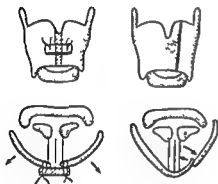
## Type I : Lateral Compression



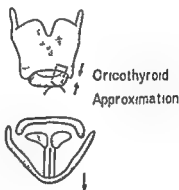
## Type III : Shortening



## Type II : Lateral Expansion



## Type IV : Lengthening



3 Four types of thyroplasty

5 Vertical incision at anterior middle third and anterior approximation of cricothyroid distance (5 in Fig 1, dogs 6 and 9)

The recurrent laryngeal nerve and the external branch of the superior laryngeal nerve on the left were cut first. The consequent glottal chink during phonation was not sufficiently eliminated by mere vertical incision at the anterior middle third of the thyroid lamina. The difference in level between the bilateral cords remained, with the left cord positioning higher than the right. The left cord was so lax that it vibrated only at the initial stage of phonation but soon remained open without vibration. The voice which started with a rough quality, soon became breathy with a widening glottal chink during phonation.

Then, the cricoid cartilage was approximated

to the thyroid by 4-0 Nylon suture just as in the case of a contraction of the cricothyroid muscle. The result was excellent: the voice was improved almost to the normal with only a slight posterior glottal slit (dog no 6) or without any slit (dog no 9).

6 Parallel vertical incision on thyroid cartilage on each side of midline and about 1 cm from it (6 in Fig 1, dog no 5)

In this case, the recurrent laryngeal nerve was not sectioned. After the parallel incisions, the median segment was depressed to slip in. The voice was much lowered in pitch but not hoarse. Pulling up and fixing the median segment back in the original position resulted in a rise of pitch up to the previous level.

## DISCUSSION

The results of the present experiment indicated that re-formation of the thyroid cartilage can quite effectively modify the position and tension of the vocal cord. Clinical indications for the techniques may therefore include insufficient glottal closure or excessive tension of the vocal cord. Treatment of dysphonia due to unilateral vocal cord paralysis, which seems most relevant to the present thyroplasty is reviewed below to evaluate the technique.

### 1 *Surgical treatment of dysphonia due to unilateral vocal cord paralysis*

In 1911, Brünings first developed a method of narrowing the glottis by paraffin injection in case of a unilateral recurrent nerve paralysis. Despite his success, this method had long been abandoned, probably because of the side effects such as paraffinoma. Recently, Arnold (1962 and 1964) and many others revived this approach with new synthetic materials such as teflon, tantalum powder, and silicone.

In 1915 Payr utilized a tilted U shaped incision on the thyroid ala to make a pedicled cartilage flap, which was then depressed inward to shift the vocal cord medially. Probably because of the pedicle, the shift effect was limited and the technique did not gain popularity.

It was Meurman (1952) who first utilized cartilage implant for this purpose. The implant taken from the costal rib was inserted between the thyroid cartilage and the inner perichondrium. Opheim (1955) used an incised segment of the thyroid cartilage instead, which was inserted inside the inner perichondrium at the level of the vocal cord. Direct intervention on the soft tissue just lateral to the vocal cord gave rise to postoperative edema, eventually requiring tracheotomy. Sawashima et al (1968) inserted an incised segment of the thyroid cartilage between the thyroid ala and inner perichondrium, with excellent results in voice. Kamei & Som (1972) inserted a piece of the thyroid cartilage from the lower rim of the thyroid cartilage in case of traumatic vocal cord paralysis.

An experimental attempt was made by Bernstein & Holt (1967) to reposition the vocal cord by inserting the bipediced vital sternohyoid muscle between the thyroid cartilage and inner perichondrium. Transposition or rotation of the arytenoid cartilage (Morrison's reverse King operation, 1948, and Montgomery's Arthrodesis, 1966) for this purpose does not seem very popular because of its technical difficulty. Mündaich (1970) pulled and fixed the arytenoid toward the lower horn of the thyroid cartilage without fenestration of the thyroid cartilage for tensing and median shifting of the vocal cord.

At present, injection of various synthetic materials appears most popular as the treatment of hoarseness in unilateral recurrent nerve paralysis. Percutaneous injection methods were recently reported by Hurst (1972) and Sato et al (1971).

As Bernstein & Holt (1967) described, there seem to be many problems left unsolved in the above treatments: the possibilities are neoplastic response or eventual rejection by the tissue of the synthetic materials, migration or absorption of the implanted tissue, local stiffening of the vocal cord due to injection and so on.

On account of the advantage that our procedure is rather simple and easy to perform and does not intervene inside the thyroid cartilage, the thyroplasty seems to have a place in treating dysphonia of vocal cord paralysis in man.

### 2 *From the functional view point thyroplasty (Isshiki) can be classified into the following 4 types (Fig. 3)*

#### Type I Lateral Compression

This type would be of widest application. Depending on the antero-posterior location and the degree of the glottal chink, incision and overlapping can be adjusted. Rather anterior incision may be used for anterior chink, and a wedge may be inserted between the overlapping cartilage laminae to reinforce the median shift of the vocal cord. An added horizontal incision or rectangular incision may also be a possibility to be considered.

### Type II Lateral Expansion

There would be few indications for this type of procedure. It also seems rather difficult to widen the angle of the thyroid cartilage. Indications for this type in hyperfunctional dysphonia such as dysphonia plica ventricularis would be a future problem to be examined.

### Type III Relaxation (Shortening) of Vocal Cord

Too high pitch as in mutational voice disorder or sulcus vocalis may be a good indication for this type of thyroplasty. Numi et al (1973) already reported that a square incision around the anterior commissure and depression of the segment was effective for treating dysphonia in sulcus vocalis.

### Type IV Stretching (Lengthening) of Vocal Cord

We attempted to lengthen the thyroid cartilage horizontally by squeezing a cartilage segment into the vertical incision crevice, but failed. For this purpose, approximation of the anterior thyrohyoid distance by mattress sutures was easier and more effective. Possible late loosening of the knot is a problem worthy of consideration.

Mixed types of the above are naturally possible. Actually, we clinically adopted a mixed type I and III for mutational voice disorder and I and IV for combined paralysis of the recurrent laryngeal nerve and the external branch of the superior laryngeal nerve. The details of the clinical cases will be reported later but the advantage is that we can select the type and adjust the extent of reformation during operation by the produced voice quality.

#### 3 Limitation of present experiment

The cartilaginous portion of the vocal cord is relatively much longer in the dog than in man. It is therefore assumed that the deficient glottal closure at the cartilaginous portion remaining after thyroplasty may be less important in the human case. Present experiments are all acute. In clinical cases of vocal cord paralysis, further consideration may be required on the problem

of accompanying vocal cord atrophy or compensatory movement of the intact vocal cord. However, the present experiment provided a fair prospect of clinical application of thyroplasty.

Together with metrological study on the human cadaver larynx now under way, clinical application has already been started. Thyroplasty as we proposed offers a new possibility in phonosurgery.

## ZUSAMMENFASSUNG

Die Frage, ob sowohl die Stellung als auch die Spannung der Stimmbänder durch die Umgestaltung des Schildknorpels verändert werden können, wurde anhand von experimentellen Tierversuchen mit 10 erwachsenen Hunden untersucht. Durch die vertikale Abschnidung des Schildknorpelflügels und die mediale Verlagerung seines lateralen Stückchens wurde die mittels Durchtrennung des N. recurrens hervorgerufene Heiserkeit im allgemeinen ausgezeichnet verbessert.

Vom funktionellen Standpunkt aus wurden 4 Typen von der Thyroplastik vorgeschlagen, die jeweils folgende Wirkung auf die Stimmbänder haben: 1. Laterale Kompression, 2. Laterale Expansion, 3. Erschlaffung (Verkürzung), 4. Spannung (Verlängerung). Ihre Operationsindikation wurde für jede Kehlkopfkrankheit gestellt. Als Vorteile der Thyroplastik beurteilen wir, dass der operative Eingriff in das Innere des Schildknorpels minimal ist, und dass daher die feine und sichere Regulierung intraoperativ ohne weiters durchgeführt werden kann.

Die Thyroplastik setzt deshalb ihre grosse Hoffnung auf die Phono-chirurgie.

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## EXTRACARDIAC RHABDOMYOMA

*Light and electron microscopic studies of two cases in the mandibular area,  
with a review of previous reports*

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(Received March 4, 1974)

**Abstract** Two cases of rhabdomyoma (one of them multifocal) in the submandibular region are described. Electron microscopic investigation of one case revealed rudimentary myofibrils which in some places showed a random arrangement, while in other parts they formed stellate figures. Hypertrophic Z bands were seen, as also were occasional A and I bands. The pathology and clinical features of rhabdomyoma are discussed on the basis of these cases and those to be found in the literature. As extracardiac rhabdomyoma is most commonly found in the region of the head and neck, it is recommended that this tumour, rare though it is, should be borne in mind in the differential diagnosis of tumours in this region.

The term rhabdomyoma is used for two types of tumour, the one cardiac and the other the far rarer extracardiac form which according to Moran & Enterline (1964) differs in its morphology. Extracardiac rhabdomyoma is considered to be a benign neoplasm of striated muscle, whereas the cardiac form is often associated with tuberous sclerosis and is more probably a developmental anomaly (Moran & Enterline, 1964).

To date, 22 cases of extracardiac rhabdomyoma have been described in the literature (Assor & Thomas, 1965; Battifora et al., 1969; Czernobilsky et al., 1968; De & Tribedi, 1940; Kay et al., 1969; Mikulowsky, 1972; Moran & Enterline, 1964; Occhipinti, 1954; Pendl, 1897; Rutz, 1926; Smith, 1959; Tandler et al., 1970; Tuazon

We report here light and electron microscopic investigations of two further cases of rhabdomyoma, both of which were localized in the submandibular region. The characteristic histological picture of this tumour makes it easy to distinguish from other tumours occurring in this area.

## CASE HISTORIES

### Case I

A 62-year-old man was admitted on 19 XI 1969 to the ENT department, (no 09 02 09-0967) Hjørring Hospital, with a pigeon-egg sized palpable tumour of the right submandibular gland. The size of the tumour had increased slowly over the course of 12 years and there had been no symptoms until the past few months, during which the patient had experienced pain and tenderness when eating. The tumour was removed in its entirety on 20 XI 1969. The surgical specimen consisted of a submandibular gland measuring 35 × 20 × 20 mm, with concretions in the duct. Microscopy revealed chronic/fibrotic sialoadenitis (due to sialolithiasis). There was nothing in the picture resembling rhabdomyoma. At postoperative examination there was an indolent soft tissue swelling. The patient was re-admitted to the department on 8 VI 1971 with a hen-egg sized intumescence in the upper part of the right side of the neck, lying in the submandibular region and extending into the lateral side of the neck. The tumour had gradually increased in size since the operation in 1969. There were no symptoms. On examination the tumour was soft, indolent, and freely mobile in relation to both skin and deep tissues. There was no enlargement of the regional lymph

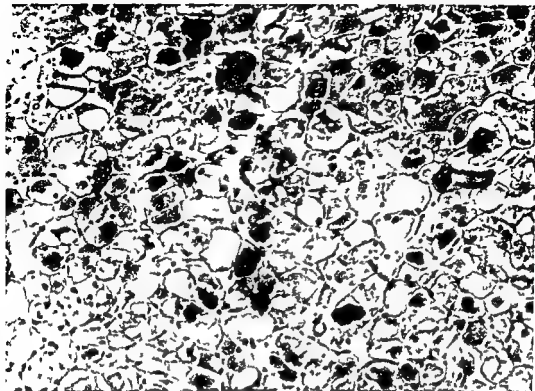


Fig. 1 The tumour is built up of large, polygonal cells with abundant cytoplasm with peripheral nuclei. The

arrangement of the tumour cells resembles that of striated muscle (case 1 haematoxylin-eosin  $\times 120$ )

findings. Other clinical and laboratory investigations (including chest X ray, haemoglobin, leucocyte and differential counts, serum glutamyl aminotransferase, aspartate aminotransferase, alkaline phosphatase, Na and K) were all normal. Operation was carried out on 9 VI 1971 with removal of a  $40 \times 30$  mm reddish brown coarsely lobulated well delimited tumour which lay deep to the parotid and extended from the upper pole of the thyroid cartilage to the angle of the jaw. Deep to the upper part of the tumour, and early separated from it, there was a  $6 \times 3$  cm tumour of similar appearance. This was also removed. At follow up 11 months after operation there was no evidence of recurrence or metastasis.

#### Case 2

A 29 year old Greenlander who had been admitted to his local district hospital 13 years previously for tuberculosis and treated with

chemotherapy for one year. The patient was admitted on 2 XI 1971 to the Department of Medicine (no. 13/72) Dronning Ingrid's Hospital, Godthaab, with a one year history of a swelling under the right mandible. The patient was aware of the presence of the tumour, but otherwise had no complaints. Examination revealed a pigeon egg sized indolent swelling immediately in front of and below the right angle of the jaw, freely mobile in relation to the skin. There was also a swelling in the floor of the mouth with displacement of the tongue towards the left. The regional lymph glands were not enlarged. Laboratory investigations (Hb, ESR) were normal. X ray of the submandibular region revealed no evidence of salivary concretions. X ray of the chest revealed evidence of old tuberculosis. On the suspicion of tuberculosis of the submandibular gland the patient given anti-tuberculous therapy, without effect. Samples of the tumour were then taken.



Fig. 2 Tumour cells showing obvious striation in the cytoplasm and peripheral nuclei. To the lower right of the picture slightly wavy, rod like crystalline structures can

be seen (arrow) (case 2, phosphotungstic acid-haematoxylin,  $\times 410$ )

scopy and for culture for tuberculosis bacillae. After the histological report of the presence of a tumour the patient was transferred to the Department of Surgery. At operation on 6 I 1972 a  $30 \times 20 \times 20$  mm, coarsely lobulated, reddish-brown, well-delimited tumour was removed. Follow-up 7 months later revealed no evidence of recurrence or metastasis.

### MATERIAL AND METHODS

On removal the tumours were fixed in 4% formalin in isotonic phosphate buffer, pH 7.4, dehydrated, and embedded in paraffin. 4–5  $\mu$ m sections were stained with haematoxylin-eosin, Van Gieson-Hansen's stain, phosphotungstic acid haematoxylin (PTAH) and periodic acid Schiff (PAS), the latter with and without digestion with diastase. In case 2, after a few days' fixation the tumour was divided into  $5 \times 2 \times 2$

mm pieces and transferred to Karnovsky's fixation fluid (1965), diluted 1:1 with distilled water.

The pieces of tumour were post fixed in 1% osmium tetroxide cacodylate buffer, pH 7.5, dehydrated first in ethyl alcohol and then propylene oxide, and embedded in Vestopal<sup>®</sup>. 1  $\mu$ m sections were cut on an LKB Ultratome and stained with toluidine blue. Ultrathin sections were made from selected areas and stained with magnesium uranyl acetate (Frasca Parks, 1965) and lead acetate (Reynolds, 1966). The slides were studied and photographed with a Siemens Elmiskop I.

### Light microscopic investigations

The tumours, which were histologically identical, were built up of large, polygonal cells with abundant cytoplasm, shaped by mutual pressure as there was little interstitial connective tissue (Fig. 1). The cytoplasm was eosinophilic

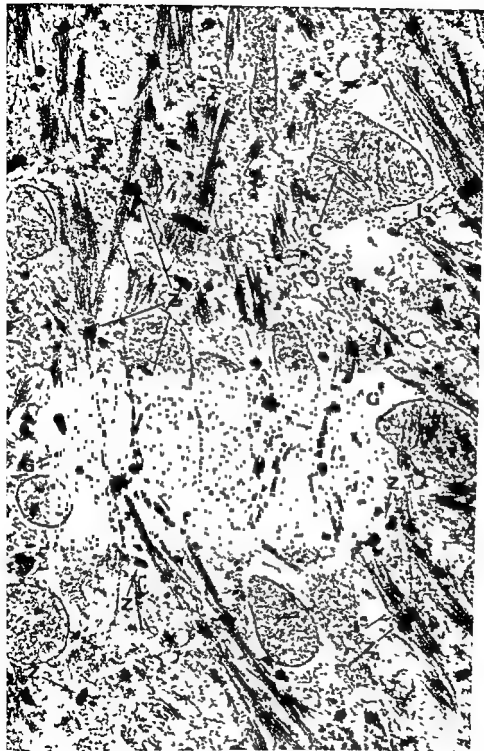


Fig. 3 Electron microscopic appearance of part of a rhabdomyoma cell. There are widespread, branching myofibrils with a periodic arrangement of very thick Z-bands (Z), which occasionally lie singly (arrow) and narrow

row pale I bands (I). There are many mitochondria, some of which show well marked cristae (C) and a number of glycogen granules (G) ( $\times 40\,000$ ).

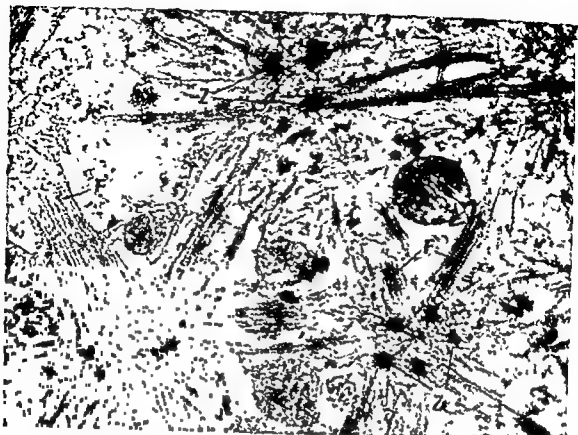


Fig 4 Electron microscopic appearance of myofibrils arranged in stellate figures with centrally placed, thick Z-bands (Z). From this the myofibrils (F) radiate out

into the cytoplasm which contains a few mitochondria ( $\times 40\,000$ )

and finely granulated, with numbers of vacuoles which were often situated in the cell periphery. Some of the cells showed transverse striations (Fig 2), which were most obvious following staining with PTAH. The nuclei often lay in the periphery of the cytoplasm, there was occasionally more than one, and there were obvious nucleoli. There was no evidence of mitosis. PAS-positive material could be demonstrated within the cytoplasm. PAS staining was negative following digestion with diastase. In case 2 it was possible to demonstrate elongated, crystal-like structures in the cytoplasm of a few of the cells (Fig 2).

#### *Electron microscopic investigations*

In the immediate neighbourhood of the tumour there were fibrils of normal striated musculature. The stroma was extremely scanty, with capillaries lined with endothelium. The tumour was

composed of cells delimited by an apparent normal double-contoured plasma membrane of varying width. There were numerous large round or oval mitochondria throughout the cytoplasm. These contained a moderately electron-dense cotton-like matrix, although in a few there were well marked cristae (Figs 3 and 4). Between these, in some cells, there were chaotically placed, frequently branching rudimentary myofibrils of varying length (Figs 3 and 4). Each myofibril was composed of parallel filaments (Fig 3). The Z-bands seemed very prominent and often showed periodic arrangement, although they occasionally lay in isolation. In a few places there were A- and I bands (Fig 3). It was also possible to find a few stellate figures composed of myofibrils which radiated out from three or more Z-bands (Fig 4).

It was not possible to distinguish between thick and thin filaments in the individual myo-

ribils. Glycogen granules were seen in a number of places. The sarcoplasmic reticulum was poorly developed and no triads composed of transverse tubuli and sarcoplasmic reticulum were seen. There were likewise no golgi complexes or larger lysosomes. The rod like crystalline structures which were described in the histological preparations were not seen in the electron microscopic preparations.

## DISCUSSION

We consider that the two tumours presented here represent benign neoplasms of the rhabdomyoma type, characterized by the well-marked delimitations of the tumour, the eosinophil granular cytoplasm, the collections of glycogen and not least, the striations.

The region of predilection for extracardiac rhabdomyoma is the head and neck, 18 of the 2 cases reported hitherto being localized in this region. The remaining tumours were found in the axilla (De & Tribedi, 1940), the thoracic wall (Occhini, 1954), the stomach (Tuazon, 1969) and the labia majora (De & Tribedi, 1940). Two tumours, as in our case 1, were multifocal (Assor & Thomas, 1969, Goldmann, 1963). These tumours have been described in patients of all ages, from 8 months to 82 years of age, without predilection for any age group, there is likewise no difference in incidence in the two sexes. In two cases the tumours were diagnosed shortly after birth both were localized to the tongue (Pendl, 1897, Rütz, 1926). In two other cases the tumour was first diagnosed at autopsy (Goldmann, 1963, Mikulowsky, 1972).

Symptoms and signs, which are completely non-specific, are exclusively dependent upon the localization and size of the tumour. The sizes of those tumours reported in the literature ranged from 7 mm to 13 cm in diameter. All tumours were well delimited, and could be removed radically. There is no report of either recurrence or evidence of malignant transformation.

Electron microscopic investigation of extracardiac rhabdomyoma (Battifora et al, 1969, Cornog & Gonatas, 1967, Kay et al, 1969, Tandler et al, 1970, Wyatt et al, 1970) reveals that the tumour reproduces the myofibril structure found in striated muscle but there is disturbance of the regular myofibril pattern. Hypertrophic Z-bands, giving rise to thin filaments corresponding to the normal I filaments, were seen in all tumours (Czernobilsky et al, 1968) while in two there were also thick filaments corresponding to the normal A-filaments (Cornog & Gonatas, 1967, Tandler et al, 1970). In the tumour described in this report it was in some places possible to demonstrate both A- and I bands, implying the presence of both thin and thick filaments. In one case (Tandler et al, 1970) all bands (Z, I, A-, H-, and M-bands) found in striated muscle have been reported, but their arrangement was random. The varying findings on electron microscopic examination indicate that rhabdomyoma may show various degrees of myofibril differentiation. The findings in embryonic muscle cells are similar, as thin filaments, are found earlier than thick filaments, and the arrangement is also random (Allen & Pepe, 1965).

In case 2 there was some degree of arrangement of the myofibrils, as in a number of places these were collected in stellate figures, this phenomenon has not previously been described, and can perhaps be interpreted as a tendency to a higher degree of organization.

Crystal like structures, described here in case 2, have been described in a number of reports, and are considered to represent hypertrophied Z bands (Battifora et al, 1969, Czernobilsky et al, 1968, Kay et al, 1969, Smith, 1959, Tuazon, 1969). We have been unable to demonstrate this correlation between light and electron microscopic findings.

As extracardiac rhabdomyoma occurs most commonly in the region of the head and it is necessary to bear this rare tumour in mind in the differential diagnosis of



## ACKNOWLEDGEMENT

The authors beg to thank the Department of Medicine, Dronning Ingrid's Hospital, for referring case 2

## ZUSAMMENFASSUNG

Zwei Fälle eines Rhabdomyoms der Regio Submandibularis (der eine multifokal) werden beschrieben. Elektronenmikroskopische Untersuchungen des einen Falls zeigten rudimentäre Myofibrillen, teils unregelmässig, teils charakteristisch sternförmig angeordnet. Hyperplastische Z-Bänder und an einzelnen Stellen auch A- und I-Bänder konnten unterschieden werden. Die Pathologie und die Klinik des Rhabdomyoms werden diskutiert. Da extrakardiale Rhabdomyome am häufigsten in der Kopf-Halsregion vorkommen, sollte dieser, obgleich sehr seltene Tumor in die Differentialdiagnose der Tumoren dieser Region mitgenommen werden.

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